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## Vocal Performance in Songbirds: Territorial Defense and the Development of Male Song and Female Mating Preferences

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**VOCAL PERFORMANCE IN SONGBIRDS: TERRITORIAL DEFENSE AND THE  
DEVELOPMENT OF MALE SONG AND FEMALE MATING PREFERENCES**

A Dissertation Presented

by

DANA LYNN MOSELEY

Submitted to the Graduate School of the  
University of Massachusetts Amherst in partial fulfillment  
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

FEBRUARY 2014

Organismic and Evolutionary Biology

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Organismic and Evolutionary Biology

## **DEDICATION**

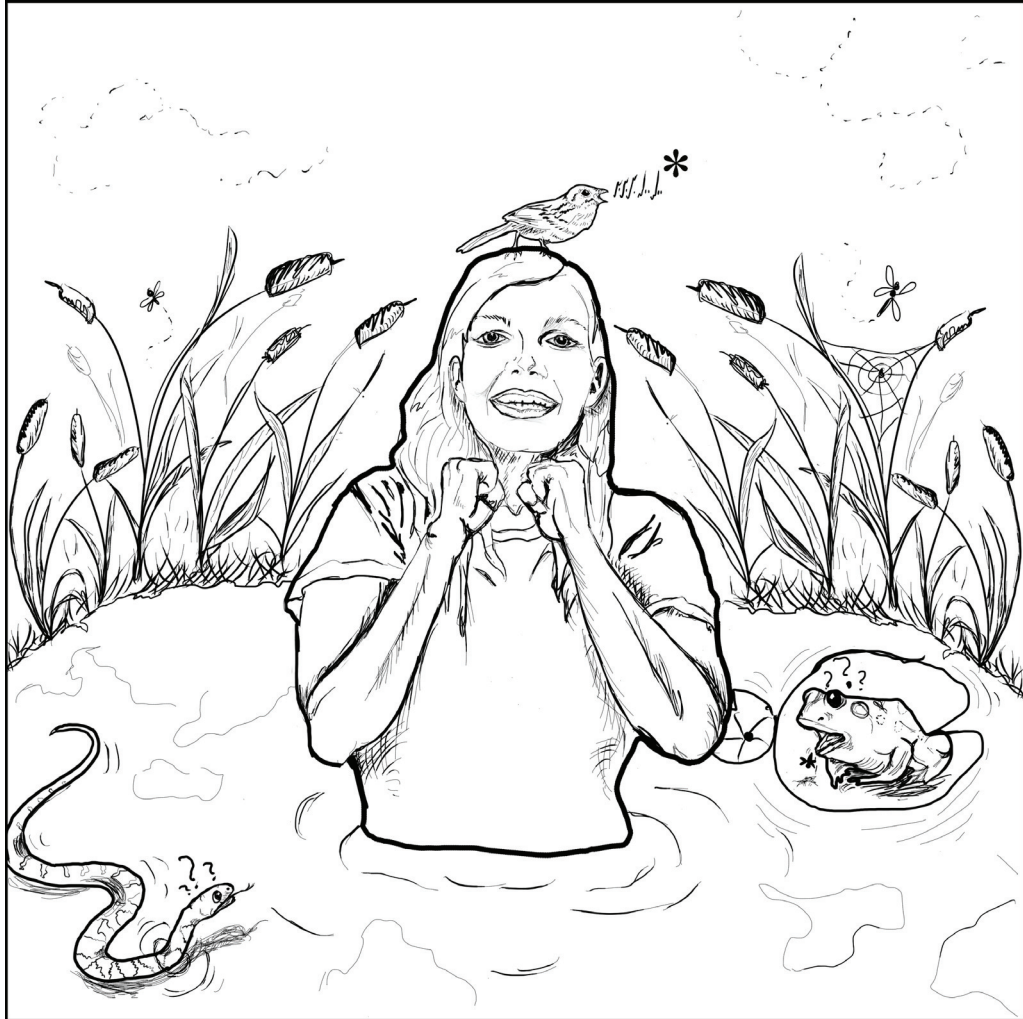
To:

My mother, Lynn Moseley, who is my role model as a scientist,  
to my loving and supportive father and sister, Phil and Lauren Moseley,  
and to the sparrows, some of whom dedicated their lives to this project.

## FRONTISPIECE

### ILLUSTRATION OF SELECTED QUOTATION I.

In long-standing tradition of the Podos Lab, the frontispiece of my dissertation includes the cartoon contest winner. Cartoons were submitted to illustrate the quotation below.



\* Roughly translates to: "Yes; though you may think me perverse, if it were proposed to me to dwell in the neighborhood of the most beautiful garden that ever human art contrived, or else of a dismal swamp, I should certainly decide for the swamp."

Quotation by Henry David Thoreau  
Illustration by Skye Long

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## ABSTRACT

### VOCAL PERFORMANCE IN SONGBIRDS: TERRITORIAL DEFENSE AND THE DEVELOPMENT OF MALE SONG AND FEMALE MATING PREFERENCES

FEBRUARY 2014

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Directed by: Professor Jeffrey Podos

The evolution of sexually selected signals has been a major topic of scientific research since Darwin. In recent years, scientists have focused on how elaborate signals can indicate honest information about the quality of their bearers, as predicted by reliability theory. A key concept relating to how mating displays could reliably reveal quality is "performance." Animals face limits in display production, and producing high-performance displays depends on the adept coordination of multiple motor systems. Thus, by observing motor performance, signal-receivers can assess the quality of signalers. Birdsong is a prime example of a display that involves motor challenges in its production. In my dissertation, I examined the connections between signal reliability and vocal performance in the swamp sparrow, *Melospiza georgiana*, and addressed three main questions. First, does vocal performance signal the level of aggressive threat during territorial defense? In wild male swamp sparrows, I measured aggressive responses to playback of various performance levels. Males responded with decreased aggression to low-performance stimuli, suggesting these stimuli indicated low threat. Males were as aggressive to control- as to high-performance stimuli, but avoided approaching high-performance stimuli as closely. Additionally, I found that males who possessed high vocal performance were more aggressive. Second, does developmental stress affect adult vocal performance? I found that birds experiencing poor early nutrition had lower vocal performance than did control birds, indicating lasting effects of early stress. Also, males in both groups significantly improved their vocal performance of learned songs between years. Together these results suggest that vocal performance can indicate early condition and age. Third, what factors influence the development of song preferences in females? In two experiments, I raised and tutored females with songs of normal performance. When tested as adults, females displayed stronger preference behavior to tutor than to novel songs, indicating the effects of learning. Females also gave the fewest displays to low-performance and responded more strongly to normal- and high-performance songs, indicating an influence of sexual selection. These experiments provide the first evidence that the development of female preference for sexually selected traits can be guided by an interplay of learning and innate biases.

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**CHAPTER 1**

**RESPONSES TO SONG PLAYBACK VARY WITH THE VOCAL PERFORMANCE OF BOTH  
SIGNAL SENDERS AND RECEIVERS**

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**Abstract**

Mating signals of many animal species are difficult to perform and thus should indicate signaler quality. Growing evidence suggests that receivers modulate their behavior in response to signals with varying performance levels, although little is known about if and how responses are affected by receiver attributes. To explore this topic I conducted two experiments with swamp sparrows, *Melospiza georgiana*, in which I challenged territorial males with playback of songs with trill rates that were natural, digitally reduced, or digitally elevated (control, low, and high-performance stimuli respectively). In the first experiment, I found that males responded more aggressively to control songs than to low-performance stimuli, that low-performance stimuli with the most severe trill-rate reductions elicited the weakest aggressive responses, and that the subjects' own trill rate significantly predicted his aggressive response. In the second experiment, I found that male responses to high-performance stimuli varied significantly, in ways predicted by two factors: the degree to which I had elevated stimulus performance levels, and subjects' own vocal performance levels. Specifically, males were less aggressive towards stimuli for which had been elevated in performance to higher degrees, and subject males with higher vocal performances themselves responded more aggressively. These findings together offer a novel illustration of how responses to aggressive signals may rely not just on signal attributes, but also on attributes of responding animals themselves.

## **Introduction**

Competitive interactions among animals are often mediated by stereotyped signals of aggression, which can help defuse interactions before they lead to direct combat (Maynard-Smith and Price 1973, Andersson 1994). The dynamics of aggressive signaling have been studied from both theoretical and empirical perspectives, and two main predictions have emerged. The first is that aggressive signals should generally provide reliable indicators of signaler attributes (e.g., resource holding potential, aggressive motivation), including the risks they represent to potential rivals. The reliability of aggressive signaling is thought to be maintained by numerous mechanisms including production constraints and receiver retaliation (Searcy and Nowicki 2005, Maynard-Smith and Harper 2003). Second, theory predicts that animals should evaluate aggressive signals with respect to their own attributes, and adjust their responses accordingly (Maynard-Smith and Parker 1976, Enquist 1985). For example in snapping shrimp, *Alpheus heterochaelis*, chelae displays are thought to signal size and fighting ability, and male responses to open chelae display models depend on the size of their own chelae relative to that of the model (Hughes 1996). Operationally it is often difficult to measure and manipulate signal values, especially when experiments pairing known opponents are not possible. Much remains to be learned about how animals modulate their responses across a spectrum of available signal values, and how receiver attributes might interact with signal values in shaping receiver responses.

Significant advances in understanding the function of aggressive signals have emerged from studies of songbirds, in which acoustic signal parameters can be manipulated with fine precision. In a majority of songbird species, males produce stereotyped songs used for both mate attraction and territory defense (Catchpole and Slater 2008). In territorial interactions, certain song parameters provoke aggressive responses, presumably because

they provide reliable indicators of threat levels posed by territorial rivals (e.g. Burt et al. 2001, Searcy et al. 2006, Hof and Hazlett 2010). In general, low-threat rivals should be relatively unlikely to provoke attack, given that they should present few risks to territorial males. For example, male great tits (*Parus major*) respond with reduced aggression to playback of songs of "losers", i.e., of simulated intruders that appeared to have lost in contests against other males (Peake et al. 2002). By contrast, I expect truly threatening intruders to induce territory holders to respond aggressively, or else to retreat.

Comparatively overlooked here is the possibility that males' responses to song are also linked to variation in their own "quality" attributes such as physiological condition, fighting prowess, and the ability to defend preferred resources such as breeding territories. I predict that territory holders of high quality would be especially likely to respond aggressively to territorial challenges. While a high-quality territory owner would likely suffer large costs if he lost a challenge, he may have sufficient fighting ability to repel most competitors. By contrast, receivers of relatively low quality might be expected to avoid or retreat from high-threat competitors to prevent costly conflict and risk of injury, even if it means giving up an established territory. Operationally, in playback studies in which high-quality intruders are simulated, one might expect to see both types of responses, with some birds attacking and others retreating (Collins 2004, de Kort et al. 2009).

In some species, the aggressive content of song – i.e., the extent to which song indicates aggressive motivation or intent – appears to be encoded in measures of trill performance (Podos et al. 2009). To elaborate, many songbirds sing trills, in which syllables are repeated in quick succession. Trills are challenging to produce because the vocal apparatus needs to be actively reconfigured during frequency modulations, and to be "reset" during brief intervals between syllables (Podos et al. 2009). Evidence for performance constraints has come from several independent lines, including observations



of tradeoffs in trill production and evolution between two acoustic parameters, trill rate and frequency bandwidth (Podos 1996, Podos 1997, Suthers et al. 2012). Thus for receivers of both sexes, trill performance may provide a reliable indicator of male quality. Supporting this interpretation, females of various species including canaries (*Serinus canaria*), swamp sparrows (*Melospiza georgiana*), and Lincoln's sparrows (*Melospiza lincolnii*) respond preferentially to trills with higher vocal performance levels (Draganoiu et al. 2002, Ballentine et al. 2004, Caro et al. 2010).

Available evidence suggests that trill performance is also attended to by rival males, but in ways that vary by species and playback context (reviewed by Podos et al. 2009). In field playback studies in which the performance of trilled stimuli varied (either naturally or through experimental manipulation) male aggressive responses to high-performance stimuli were diminished in red-winged blackbirds (*Agelaius phoeniceus*) (Cramer and Price 2007), yet elevated in banded wrens (*Thryothorus pleurostictus*), at least in subjects' initial reactions (Illes et al. 2006). Schmidt et al. (2008), working with European nightingales (*Luscinia megarhynchos*), reported results parallel to those for banded wrens (Illes et al. 2006), and also observed that males that remained unpaired during that breeding season showed low responses to fast trill playback, whereas males that did later pair with females maintained aggressive responses (Schmidt et al. 2008). In another playback experiment on banded wrens, however, males were found to respond less aggressively to high-performance songs than to medium-performance songs (de Kort et al. 2009a). These studies and others suggest that high performance trills elicit wide-ranging responses, varying from increased aggression to avoidance. We know little about why some individuals respond to high-performance songs with aggression, while others respond with avoidance or retreat.

The present study focuses on the swamp sparrow, a species in which males sing repertoires of trilled songs. Vocal performance can be assessed readily by measuring trill

rate and frequency bandwidth, and trills for playback presentation can be digitally manipulated to various performance levels without changing other song features (Podos 1996, 1997, Lahti et al. 2011) (Fig. 1.1). Prior work with swamp sparrows indicates that males discriminate among songs with varying vocal performance levels, giving stronger flight and vocal responses when presented with song types of higher versus lower performance (DuBois et al. 2011). Additionally, males tend to slightly elevate vocal performance when presented with simulated territorial intrusion (DuBois et al. 2009), although at levels that are not detected by other males in field conditions (DuBois et al. 2011).

I hypothesize that males respond to variation in vocal performance in accordance with the level of threat indicated thereby, and further that responses also vary with males' own vocal performance. To test this hypothesis I presented territorial males with playback of control songs and test songs in which trill rate, and thus perceived vocal performance, was digitally manipulated either to low (Experiment 1) or high performance levels (Experiment 2). For Experiment 1 I predicted that responses would be greater to control songs than to low-performance songs, the latter of which should represent universally low levels of threat. For Experiment 2 I predicted that responses to high performance stimuli would vary depending on the vocal performance levels of both playback stimuli and the subject males. More specifically, I predicted that stimuli with performance levels increased slightly would be responded to aggressively, while stimuli increased to the highest performance levels would be avoided, because of the higher perceived risk. Further, for both experiments, I predicted that subjects' tendencies to engage simulated intruders would vary positively with their own vocal performance.

## **Methods**

### **Study Site and Subjects**

This study was conducted in a marsh habitat on the Prescott Peninsula of the Quabbin Reservoir in Franklin County, western Massachusetts, USA (42° 25' N, 72° 20' W). Swamp sparrows established territories of roughly 400-600 m<sup>2</sup> in late April and early May, and actively defended these territories through late July. Beginning in 2005 I captured birds with mist-nets and gave males unique color and FWS aluminum band combinations (IACUC 28-10-02).

I mapped territories by observing and noting locations of males and females as they fed, perched, sang, and foraged. For a majority of study subjects I determined nest locations, and for all subjects estimated breeding status. By estimating breeding status I was able to conduct playback trials during a common stage, before initial clutches or between successive clutches.

### **Song Recording and Vocal Performance**

Between 2005 and 2010 I compiled a library of song recordings from males in the local population, made using Marantz PMD660 digital recorders and Sennheiser ME66 directional microphones. Swamp sparrows sing a modest repertoire of two to five songs types, which can be classified by the number and category of notes in a syllable (Marler and Pickert 1984). Spectrograms of recordings were reviewed on Audacity (<http://audacity.sourceforge.net>) to classify songs. For each male, the song type most commonly produced during playback trials was used to calculate his vocal performance (DuBois et al. 2009, 2011).

For songs selected as playback stimuli, and for songs recorded from study subjects (n=3-5 renditions per bird), I estimated vocal performance using measurements of trill rate (the average rate of syllable repetition in a song in Hz) and frequency bandwidth (the range

of frequencies represented in the song in KHz) with the program SIGNAL (Engineering Design 2003). Trill rate was measured across 8 syllables in the middle of each song, and frequency bandwidth was calculated from amplitude spectra using a threshold of -24 dB relative to the peak amplitude (Podos 1997). A metric of vocal performance, “vocal deviation”, was calculated as in Podos (2001); the average trill rate and frequency bandwidth for each song type was plotted, and the distance of each point to the upper bound regression for emberizids ( $y = -0.124x + 7.5$ , Podos 1997) was calculated. Greater vocal deviation values represent lower performance, whereas smaller deviations represent higher performance (Podos 1997, Ballentine et al. 2004) (Fig. 1.1, Table 1.1). With this method each male and playback stimulus was assigned a vocal performance score.

### **Experiment 1: Response to low- versus control-performance songs**

The goal of my first experiment was to compare aggressive responses to low- versus control-performance songs. To prepare experimental stimuli, I selected songs from high quality recordings from 11 males of known identity. Each song used to construct a playback stimulus was a unique combination of singer and song type, with no song used more than once. These songs varied in the number and category of notes per syllable, and represented natural variation in swamp sparrow song across the population.

Next I created a "control" and a "low" performance version of each of the 11 songs, resulting in 22 playback stimuli. All playback stimuli were constructed by concatenating a single syllable into a repeated sequence of syllables (a song) of two seconds in duration, following previous methods (Lahti et al. 2011, Podos 1996, see Fig. 1.1). In constructing control-performance stimuli I preserved the original wild-recorded syllable timing and trill rate. Control songs ranged from 4.35-10.61 Hz in trill rate, and from 1.82-3.19 in vocal deviation. Low-performance stimuli were constructed by digitally adding silent intervals between notes and syllables proportionally, thus adjusting syllable timing while preserving

the original structure of individual notes (as in Lahti et al. 2011). Songs were slowed to 35-80% of their original trill rates, resulting in stimuli ranging from 2.19-7.49 Hz in trill rate, and 2.20-3.76 in vocal deviation. All stimuli were standardized to the same maximum amplitude, and played in trials at one song per ten seconds for three minutes, which approximates the natural singing rate for this species (Ballentine et al. 2004, DuBois et al. 2009, 2011).

### **Playback Trials**

Playback trials were conducted during June and July of 2009 and 2010. Each bird received two trials, and each trial consisted of either the control- or the low-performance version of the same song type. Song stimulus sets were selected randomly from banded males that were unfamiliar to the subject males, either recorded from a male out of earshot or from a male only present in a year in which the subject male was not. The order of presentation for control- versus low-performance was determined using a balanced design.

Each subject's two trials were separated by 1-3 days, and were conducted between 0600-1200 h. I placed a portable field speaker (SME-AFS speaker, Magnavox CD player) just within a subject's territory, in a bush or fixed to a tree at roughly 1.5 m height, in the same location for both trials. Two observers were present at all trials (DLM and DCL) to ensure continuous observation of playback subjects. One observer dictated response behaviors and recorded subjects' vocalizations with a Marantz digital recorder, while the other observer dictated and filmed behavior with a digital video camera (Canon Handycam) from a distance of about 10 m from the speaker.

Before each trial, I located the subject male within the territory and recorded his behavior. I recorded, filmed, and annotated the behavior of subjects during the three-minute stimulus playback as well as for an additional seven minutes post-playback. Response behaviors during the ten-minute block recorded included the production of

broadcast and soft songs, flights, passes within 2 m of the speaker, closest approach to the speaker, and latency to first song. The two observers conferred on distance estimates before and after each trial, and compared and reconciled differences between observations.

### **Statistical Analysis**

Responses to control- versus low-performance stimuli were quantified on a per-response variable basis, and then reduced using principal components analysis (PCA) to one overall measure of aggressive response, PC1. I used the raw and PC1 response scores for each male to compare responses between low- and control-performance trials, using Wilcoxon signed ranks tests (JMP SAS and R).

I then conducted multiple regression analysis with model selection, in which I regressed subjects' overall aggressive responses to low-performance trials (PC1 scores) against selected predictor variables. I first tested my manipulation of the playback stimuli alone as a predictor, and then tested all possible models including variables relating to the subject males. This analysis aimed to test whether quantitative variation in the stimulus manipulation would have a systematic effect on birds' responses (for a parallel analysis see Lahti et al. 2011), and whether birds' own vocal performance levels predicted their responses to the playback stimuli. I evaluated the models using Akaike's information criteria (AIC), to identify the model with the best fit.

### **Experiment 2: Response to high- versus control-performance songs**

The goal of my second experiment was to test levels of threat signaled by high- versus control- performance songs. I predicted that males would respond more or less strongly to such stimuli, depending on the extent to which I increased the performance level of playback songs. The study subjects were 19 male swamp sparrows, none of which were used in Experiment 1. Experiment 2 was conducted in June and July of 2006-2008, and followed the methods of Exp. 1 except as indicated below.

I created 19 sets of "control" and "high" performance stimuli, and each subject male received a unique song type by singer combination. I prepared control stimuli as in Exp. 1, using wild-recorded songs that ranged from 4.75-9.48 Hz in trill rate and 1.96-3.31 in vocal deviation. To create high-performance stimuli, I increased the trill rate by deleting silent intervals between notes and syllables proportionally, following procedures used previously (Podos 1996, Podos et al. 1999). Songs were increased to 115 - 155% of their wild-recorded rate, which resulted in high performance stimuli with trill rates of 7.0-14.1 Hz and vocal deviations from 1.39-3.03. Each subject received two trials, one song type at control and one at high performance.

In addition to the response variables of Exp. 1, I also measured the time subject males spent within 1m or 3m of the speaker, to account for my observation that some males tended to flee or avoid the speaker location, while others made aggressive approaches and stayed in close proximity. Because some males fled during the initial stimulus period but returned later, I calculated broadcast song and flight rate for multiple time blocks including the 3 min playback period and the total trial period, and I calculated the post-playback period for song rate as males tended to increase song rate after cessation of the stimulus.

### **Statistical Analysis**

As in Exp. 1, I initially examined differences between the two stimulus categories, control- and high-performance, for each response variable. My next goal was to test the effect of both the stimulus manipulation and the subject males' vocal performance on response strength. Since some males responded more strongly to control stimuli, while others clearly responded more strongly to high-performance stimuli, I determined whether males were more aggressive or avoiding of the high-performance stimulus as compared to the control. To do so I calculated, for each response variable, the difference between each male's response to high-performance and his response to control stimuli – positive values

always indicated stronger response (more aggression) to high performance, while negative values indicated stronger response to control. I reduced these response differences with PCA, generating three principal components (Table 1.3). I ran multiple regression analysis with model selection using PCs 1-3 factor scores as the response variables, first testing the effects of the stimulus manipulation alone and then all variables together (listed in Table 1.5). Finally I evaluated the explanatory power of all variables using model selection with AIC, comparing all combinations of predictor variables and identifying the best-fit, but not over-fit, model.

## **Results**

### **Experiment 1: low- versus control-performance trials**

Males responded less strongly to low- than to control-performance stimuli. All six raw response variables followed the same pattern of being weaker during playback of low- versus control-performance stimuli (Fig. 1.2), with the difference across treatments in soft song rate achieving significance (Wilcoxon signed ranks test,  $Z=2.72$   $p=0.004$ ). In PCA, all six raw response variables loaded positively and evenly (loadings between 0.35-0.44) onto PC1, which explained 55.9% of the overall variation. I found a significant difference in PC1 scores by condition, with higher responses to control- than to low-performance stimuli (Wilcoxon signed-ranks test,  $n=11$ ,  $Z= -2.66$ ,  $p=0.008$ , Fig. 1.2).

When testing the stimulus manipulation alone, the regression analysis revealed a significant effect of stimulus vocal performance on male aggressive response (Table 1.2). The best-fit model in the multiple regression was determined to include variables relating to the stimuli and a component of the subject males' vocal performance, trill rate. The best-fit model included as predictor variables the percent trill rate manipulation, the difference in vocal deviation between the low and control-performance stimuli, and subject males' trill



rates (best-fit model  $R^2 = 0.868$ ,  $n = 11$ ,  $df = 3, 7$ ,  $F = 15.38$ ,  $p = 0.002$ ,  $AIC = 1.78$  vs 3.4 and higher, see also Table 1.2). Specifically, the more I reduced stimulus trill rate from the wild-recorded rate, the less aggressive males were in response (Fig. 1.3a). Additionally, subject male trill rate was included as a positive predictor of aggressive response (Fig. 1.3b, Table 1.2), indicating higher male trill rates tended to correspond to more aggressive responses. Results were robust to sequential-Bonferroni correction for multiple tests.

### **Experiment 2: high- versus control-performance trials**

On average males appeared to respond as aggressively to control as to high-performance stimuli (Fig. 1.2), but males varied individually as to which stimulus they responded to more aggressively. Specifically, males responded to high-performance trials with either elevated aggression or with diminished aggression and avoidance. Differences in responses reduced to three principal components and cumulatively explained 74.7% of the variation (Table 1.3). Singing behaviors (songs, soft-songs) loaded most strongly onto PC1, flying behaviors (flights, passes) onto PC2, and approach behaviors (closest approach, time spent close to the speaker) onto PC3. Another variable that loaded somewhat strongly on PC3 was the rate of singing after cessation of the playback stimulus.

In the multiple regression analysis with model selection, models with PC1 or PC2 as response variables were not significant ( $p > 0.05$ ), but models testing PC3 were significant (Table 1.4). In model selection analysis for PC3 (approach, time spent close), the best-fit model was determined to be the full model and included predictor variables of both playback stimuli and subject males (Table 1.5,  $n = 19$ ,  $R^2 = 0.725$ ,  $F = 4.15$ ,  $p = 0.018$ ,  $AIC = 3.23$  vs 4.06 and larger;  $SSE = 9.7$ ). There was a significant negative effect of the percent stimulus manipulation indicating that higher stimulus performance levels were associated with males staying farther away and spending less time close to the speaker (Fig. 1.3c). For predictor variables relating to the subject males there was a significant positive effect; the

higher a male's own vocal performance (high trill rate and frequency bandwidth), the more likely he was to approach and spend time closer to the speaker during high-performance stimulus playback (Fig. 1.3d).

### **Discussion**

As expected, responses to song playback in both experiments varied with stimulus attributes. In the first experiment, territorial male swamp sparrows responded significantly less strongly to low-performance than to control-performance playback stimuli, consistent with my hypothesis that receivers should attribute limited threat to low-performance songs (Fig. 1.2). Additionally, males' reduced aggression to low-performance stimuli was more pronounced for playback stimuli that had been lowered further from their control trill rates (Fig. 1.3a). Notably, males sang significantly fewer soft songs to low performance stimuli (Fig. 1.2); singing softly in response to simulated territorial intrusion is known to be a highly reliable predictor of attack in swamp sparrows (Ballentine et al. 2008), song sparrows (*Melospiza melodia*) (Searcy et al. 2006), and black-throated blue warblers (*Setophaga caerulescens*) (Hof and Hazlett 2010). This suggests that males would be more likely to attack intruders singing control- versus low-performance songs.

In my second experiment, high performance stimuli elicited aggressive responses in some individuals, yet avoidance behavior in others, resulting in similar overall outcomes across treatments (Fig. 1.2). Subjects' tendencies to respond one way or the other varied consistently with performance levels of playback stimuli. Specifically, I found that birds did not approach as closely or remain near the speaker during presentation of the stimuli that I had manipulated to the greatest extent, as measured by the percent increase of stimuli above their original trill rate (Table 1.5, Fig. 1.3c). Closest approach was previously indicated as a reliable predictor of attack in swamp sparrows (Ballentine et al. 2008). Prior

studies in banded wrens, red-winged blackbirds, and nightingales showed differential male approaches to high-performance playback, which either deterred or provoked aggressive approach by subjects (Illes et al. 2006, de Kort et al. 2009a, Cramer and Price 2007, Schmidt et al. 2008). Similar to DuBois et al. (2011), I found that swamp sparrows responded less to low-performance stimuli and responded strongly to stimuli ranging from control to slightly increased trill rates (higher performance). While their study tested songs within average inter-male differences, I expanded on this by testing a continuum of vocal performance levels, pushed to low and high extremes, in order to determine factors that would predict aggression versus avoidance particularly in response to high performance.

A key additional finding in both my experiments was that responses varied in accordance with the vocal performance of study subjects themselves. In Exp. 1, birds' tendencies to respond with greater strength was predicted by their own trill rates, with birds singing higher trill rates tending to respond comparatively more aggressively to playback (Fig. 1.3b, Table 1.2). In Exp. 2, subjects that possessed higher vocal performance levels approached high-performance stimuli more closely and spent more time close to the playback speaker, whereas subject males singing lower performance songs remained at a farther distance from the playback speaker (Table 1.5, Fig. 1.3d).

How variation in subject attributes (e.g., vocal parameters, overall quality) shape responses to territorial playback has been virtually overlooked in studies of signal function in songbirds. My data suggest that subject attributes can be important predictors of responses to territorial intrusion. Available evidence from several songbird species indicates that vocal performance can correlate with age, size, or other quality attributes (e.g. Ballentine et al. 2009, de Kort et al. 2009b), and males with high performance songs would therefore be better positioned to ward off the challenge of high-performance intruders. In agonistic contests, the importance of subjects' own attributes as predictors of behavior has

been established in other taxa (Elwood and Arnott 2012), but fewer studies have shown the connection to receiver signal values specifically (e.g. Hughes 1996, Crothers et al. 2011). For example, in a study of hermit crabs (*Pagurus berhardus*), model intruders that signaled aggressively were seen to elicit varied responses, with the likelihood of different responses (attack or retreat) depending on whether study subjects had initially signaled with threat displays themselves (Laidre 2009). Focusing on signal values specifically, a recent study of poison frogs, *Dendrobates pumilio*, showed that males responded more aggressively (quicker approaches and more frequent calls) to stimulus frogs with brighter dorsal colors, and moreover subjects' own brightness predicted their aggressive calling and approach (Crothers et al. 2011). Parallels between these studies and my results supports the generality of the interpretation that receivers with high signal values themselves are more aggressive in their responses to opponents, especially those that are highly threatening.

Returning to my data, I offer a possible explanation for the main difference between the two experiments, i.e., why average response scores differed between control- versus low-performance stimuli but not between control- versus high-performance stimuli. On the one hand, disregarding low performance signals should not carry high costs for any males, even those who themselves are of low quality. Males on territories would likely suffer little impact on their reproductive success if they were to tolerate younger or poorer quality males on their territories, as it is unlikely that females would select males with low-performance songs for extra-pair mating (Ballentine et al. 2004, 2009, Searcy et al. 2010, Byers et al. 2010, Moseley unpub. data). It thus may not be surprising that responses to low-performance stimuli were uniformly low. On the other hand, failing to respond to high-performance signals, even ones that push the bounds of species-typical acoustic structure, could readily result in the loss of a territory to a higher quality opponent (Searcy and Brenowitz 1988), in the loss of paternity from eavesdropping by females and other rivals

(Mennill et al. 2002), or in direct conflict. If a perceived threat is too great, however, territorial males may benefit from avoiding such costly conflicts (Hof and Hazlett 2012), and I often observed that the immediate response of some males to some high-performance stimuli was to flee.

An important assumption I am making in interpreting my data is that males are able to recognize the performance levels of song stimuli, and to modulate their responses accordingly. An alternative possible explanation for reduced responses to manipulated songs is that males failed to recognize such songs as conspecific. Indeed a few of my manipulated stimuli featured trill rates below 4.3Hz and beyond 12.5Hz, outside of swamp sparrows' natural range, of trill rates (although not vocal deviations), but these made up less than one-third of all stimuli. However, this alternative explanation is countered by song learning studies which have shown that male swamp sparrows perceive and successfully memorize songs manipulated to much higher and lower performance levels, treating these songs as viable learning models (Podos 1996, Podos et al. 1999, Lahti et al. 2011). Hand-reared male swamp sparrows were found to be able to memorize with precision song models of extremely high-performance levels (trill rates increased to 160-187%) in the early sensory-motor phase (Podos 1996, Podos et al. 1999). At the other end of the spectrum, young male swamp sparrows were found to be able to learn song models reduced to as low as 55% of their original trill rates, albeit with reduced copying accuracy (Lahti et al. 2011). Also consistent with my inference that all stimuli were recognized as conspecific is the observation that in the present study males responded with some aggressive behavior, including broadcast songs and passes in close range of the speaker, even to stimuli that were altered considerably, e.g. to 35% or 155% of the original trill rate. In Exp. 1, none of the 11 males completely ignored the low-performance stimuli, but instead responded with some aggressive singing, flying, and approach behavior. I thus infer that

low-performance songs were recognized as relevant, yet did not represent threats sufficient to elicit strong responses (Searcy et al. 2006, Ballentine et al. 2008). In Exp. 2, as mentioned above, some individuals reserved their most aggressive responses for high-performance stimuli. It is also notable that males possessing songs of lower performance, that were initially deterred during the high-performance speaker, often returned after playback ended, and then sang at rates comparable to those of other males that had responded strongly during playback. This recovery of responses is consistent with behaviors of males interacting with a conspecific intruder (Catchpole and Slater 2008).

Taken together, my results provide a novel line of support for the hypothesis that vocal performance provides a reliable signal of aggressive threat. Searcy and Nowicki (2005) outlined three criteria needed to demonstrate that a signal is reliable: (1) some aspect of the signal must correlate with signaler attributes; (2) receivers should respond to the signal in ways consistent with its presumed function; and (3) receivers should respond in ways that provide them with overall fitness benefits. Considering the potential reliability of vocal performance in songbirds, the first criterion had been addressed in prior studies, including in swamp sparrows (Ballentine et al. 2009, de Kort et al. 2009b). These studies showed that older or larger males tend to sing at higher vocal performance levels, thus presumably presenting greater threats to territorial rivals. Similarly in my study, higher vocal performance or trill rate correlated with increased approach and aggression (Fig. 1.3b,d). This helps to explain why males with higher vocal performance responded more aggressively to high-performance stimuli. My data also support the second reliability criterion, by showing that male swamp sparrows respond to playback stimuli in degrees consistent with presumed levels of threat (low responses to low performance and aggressive or avoidance behavior in response to high performance). Finally, although not tested explicitly here, the data are consistent with the third criterion. Low-quality males

should benefit by retreating from high-threat signals to avoid the risk of costly combat, whereas high-quality males should benefit by being aggressive, so as to limit the risk of losing a territory or paternity. The potential fitness benefits of singing higher performance songs could be assessed most directly in field studies of territorial males, in which vocal behavior, territorial interactions, and breeding success are all quantified.

Most broadly, my data contribute to a general understanding of how animals respond to signals or signalers that are threatening. Traditionally, researchers predicted that playback of threatening signals should result in defensive responses proportional to the level of threat signals represent (e.g. Falls et al. 1982), a prediction supported in empirical studies including some that have focused on trilled vocalizations (Illes et al. 2006). More recent studies, however, report circumstances in which high-performance signals elicit less of an aggressive response than do signals of lower or more intermediate levels of performance (de Kort et al. 2009a, Cramer and Price 2007). A possible explanation for this outcome is that intruders signaling at particularly high threat levels might present too great a risk for a territory holder to engage in conflict (Collins 2006, de Kort et al. 2009a). My study extends this suggestion by showing that birds' responses to high-threat stimuli correspond not just to the degree of threat presented by an intruder, but also to their own vocal performance, as predicted more generally by Maynard Smith & Parker (1976). We do not yet know if this correspondence is best explained as self- or mutual-assessment (Elwood and Arnott 2012), nor do we know whether there is an underlying physiological relationship between vocal performance and male attributes such as resource holding potential. In Fig. 1.4 I present a schematic model that summarizes male responses to territorial intrusion (y-axis) as it varies with both factors (see legend for more detail). One could potentially test this model using a factorial design that presents subject males of

known vocal performance with playbacks of much higher and much lower vocal performances.

To conclude, responses of territorial males to varying vocal performances of rivals correlate not just with threat levels posed by intruders (as shown in prior studies), but also by subjects' own vocal performance capabilities (novel to this study). Researchers seeking to identify signaling traits that mediate aggressive contests thus should take into consideration not only the properties of signal features, but also the individual quality and signaling capabilities of their study subjects, and potential interactions among signaler and receiver attributes.



Table 1.1 Details of stimuli sets used in Experiment 1 (n=11) and 2 (n=19). Each stimulus set included one song type at a control and at a manipulated trill rate.

<b>No. of notes/ syllable</b>	<b>% of original</b>	<b>control trill rate (Hz)</b>	<b>modified trill rate (Hz)</b>	<b>frequency bandwidth (kHz)</b>	<b>control vocal deviation</b>	<b>modified vocal deviation</b>
<b>Exp. 1</b>						
3	70	10.61	7.49	4.400	1.82	2.20
3	60	8.49	5.20	3.499	2.98	3.55
4	35	6.24	2.19	3.836	2.92	3.42
3	40	9.50	3.79	4.500	1.86	2.55
4	50	9.50	4.75	3.965	2.39	2.97
2	45	9.48	4.26	4.273	2.09	2.73
4	65	7.57	4.92	3.898	2.69	3.02
5	75	4.35	3.26	3.797	3.19	3.32
4	40	7.30	2.92	3.662	2.96	3.76
3	80	6.30	5.00	3.797	2.95	3.11
4	55	7.94	4.36	3.800	2.74	3.18
<b>Exp. 2</b>						
3	147.5	7.10	11.00	3.763	2.88	2.40
3	122	7.50	8.00	3.595	3.00	2.94
3	151	9.50	14.00	3.058	3.29	2.74
3	155	7.79	12.07	4.161	2.40	1.88
5	147.5	4.75	7.00	3.629	3.31	3.03
4	120	6.56	7.87	4.228	2.49	2.33
4	125	8.53	10.67	4.261	2.21	1.95
4	140	7.45	10.42	3.667	2.94	2.57
3	150	6.62	9.93	4.200	2.51	2.10
4	115	8.33	9.58	3.362	3.13	2.58
4	142.5	7.33	9.70	3.662	2.96	2.66
3	135	6.26	8.45	3.797	2.95	2.68
4	132.5	6.06	8.03	3.768	3.01	2.76
5	127.5	6.73	8.75	3.668	3.03	2.78
4	142.5	8.20	11.69	4.061	2.45	2.02
3	145	7.58	10.99	3.467	3.12	2.70
4	130	6.19	8.04	3.937	2.82	2.60
3	149	9.48	14.1	4.400	1.96	1.39
2	120	9.50	11.4	4.273	2.08	1.85

Table 1. 2 Experiment 1: Multiple regression analyses of playback performance level on male aggressive response. I first tested the manipulation of the playback stimuli alone, and the percent of the original trill rate significantly predicted males' overall aggressive response to playback in the low performance trial (PC1). In other words, the further the stimulus was reduced from the original rate, the less aggressively males responded ( $R^2 = 0.368$ ,  $n = 11$ ,  $df = 10$ ,  $\beta = 0.0864$ ,  $F = 5.25$ ,  $p = 0.0477$ ). Additionally when tested alone, subject males' own trill rate significantly predicted the aggressive response (PC1), with males possessing higher trill rates responding more aggressively ( $R^2 = 0.392$ ,  $n = 11$ ,  $df = 10$ ,  $\beta = 1.457$ ,  $F = 5.796$ ,  $p = 0.0394$ ).

Through model selection, the best-fit model ( $R^2 = 0.868$ ,  $n = 11$ ,  $df(\text{model}) = 3$ ,  $df(\text{error}) = 7$ ,  $F = 15.38$ ,  $p = 0.002$ ,  $AIC = 1.78$ , compared to 3.4 and higher) was determined to include the following variables below. All predictor variables tested were as follows, stimuli: % of original trill rate, trill rate of low performance, and difference in vocal deviation between original and low performance; subject males: trill rate, frequency bandwidth, vocal deviation.

<b>Predictor Variables of</b>				
<b>best-fit model</b>	<b><math>\beta</math></b>	<b>SS</b>	<b>F</b>	<b>p</b>
% of original trill rate of playback	0.21	22.57	25.27	0.0015
difference in stimuli vocal deviation	11.70	15.55	17.42	0.0042
trill rate of subject male	0.82	4.81	5.38	0.0534

Table 1. 3 Principal component analysis of response differences between playback of high and control performance measured in Exp. 2. Values reduced by PCA were the result of high minus control responses for all variables except closest approach and latency, for which I did the opposite subtraction, so that a larger number would similarly indicate a more aggressive response to the high-performance trial. PCA generated three PCs on which loaded singing variables (PC1), flying variables (PC2), and approach/duration variables (PC3) with Eigen values of 3.4 through 2.0.

<b>Principal Component</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>
Percent	31.0	25.9	17.8
<b>Eigen vectors</b>	<b>Loadings</b>		
initial songs	<b>0.50</b>	0.00	0.13
total songs	<b>0.48</b>	0.03	0.24
post-stimulus songs	<b>0.40</b>	0.04	<b>0.37</b>
soft songs	<b>0.35</b>	0.16	0.03
latency to first song	<b>0.38</b>	0.10	0.03
initial flights	0.04	<b>0.50</b>	0.23
total flights	0.09	<b>0.53</b>	0.04
passes	0.04	<b>0.52</b>	0.08
time within 1 meter	0.22	0.21	<b>0.50</b>
time within 3 meters	0.18	0.22	<b>0.55</b>
closest approach	0.05	0.25	<b>0.41</b>

Table 1. 4 Experiment 2: Multiple regression analyses did detect significant results for response differences for PC3 (closest approach, time close to the speaker) including only variables of the stimulus manipulation. The degree of the playback manipulation significantly predicted subject male aggressive response, the higher the performance of the playback stimuli, the lower the aggressive response ( $R^2 = 0.50$ ,  $n = 19$ ,  $df(\text{total}) = 18$ ,  $df(\text{model}) = 4$ ,  $F = 3.48$ ,  $p = 0.036$ ). Significant predictor variables within the model were the % of trill rate increase as compared to the control-rate and the interaction between the trill rate of the high performance stimuli by the notes/syllable (a measure of trill performance). Multiple regression models with PC1 singing or PC2 flying as response variables were not detected as significant in model evaluation, PC1 ( $F=0.468$ ,  $p=0.758$ ), PC2 ( $F=0.881$ ,  $p=0.5$ ).

<b>Predictor Variables of playback</b>	<b><math>\beta</math></b>	<b>SS</b>	<b>F</b>	<b>p</b>
% trill rate increase	<b>-0.07</b>	9.05	<b>7.15</b>	<b>0.018</b>
trill rate (Hz)	0.23	2.13	1.68	0.216
# of notes/syllable	0.25	0.42	0.33	0.574
trill rate (Hz) x notes/syllable	<b>-0.59</b>	9.95	<b>7.87</b>	<b>0.014</b>

Table 1. 5 Experiment 2: Multiple regression analysis of variables predicting the direction and degree of response to high- versus control-performance stimuli. Results determined by model selection (AIC) indicate the full model as best-fit, which includes the vocal performance of both playback stimuli and subject males as predictor variables for PC3 Approach/Duration ( $R^2=0.725$   $n=19$ ,  $df=18$ ,  $F=4.15$ ,  $p=0.018$ ). Asterisk (\*) denotes individual variables that achieved significance within the model. See text and Fig. 1.3c,d for further explanation.

<b>Predictor Variables</b>	<b><math>\beta</math></b>	<b>SS</b>	<b>F</b>	<b>p</b>
<i><u>Variables of the playback stimulus</u></i>				
% trill increased	-0.07	8.57	9.71	<b>0.010</b>
trill rate	0.39	5.08	5.76	<b>0.035</b>
# of notes per syllable	0.81	3.39	3.85	0.076
trill rate x notes/syllable	-0.28	1.63	1.85	0.201
<i><u>Variables of the Subject Male</u></i>				
vocal deviation	274.3	7.12	8.08	<b>0.016</b>
trill rate	31.0	7.01	7.95	<b>0.017</b>
frequency bandwidth	0.27	7.11	8.06	<b>0.016</b>

## **Chapter 1 Figures**

Figure 1. 1 Example spectrograms of playback stimuli used in control and manipulated performance trials (upper panel), and frequency bandwidth (kHz) plotted as a function of trill rate (Hz) for stimulus and subject songs (below).

Upper Panels: Each subject was presented with two versions of one song type, one at its natural trill rate (left column) and the other version at either a reduced trill rate (Exp. 1, right column rows a & b) or an elevated trill rate (Exp. 2, right column rows c & d).

Lower Panel: The family-wide "upper-bound regression" (line of descending slope), from which "vocal deviation" values are calculated, is provided for reference (see text and Podos 1997). Plotted are control stimuli and songs from subject males (circles), low-performance stimuli (triangles, Exp. 1), high-performance stimuli (diamonds, Exp. 2). Open symbols correspond to the 8 spectrograms shown above.

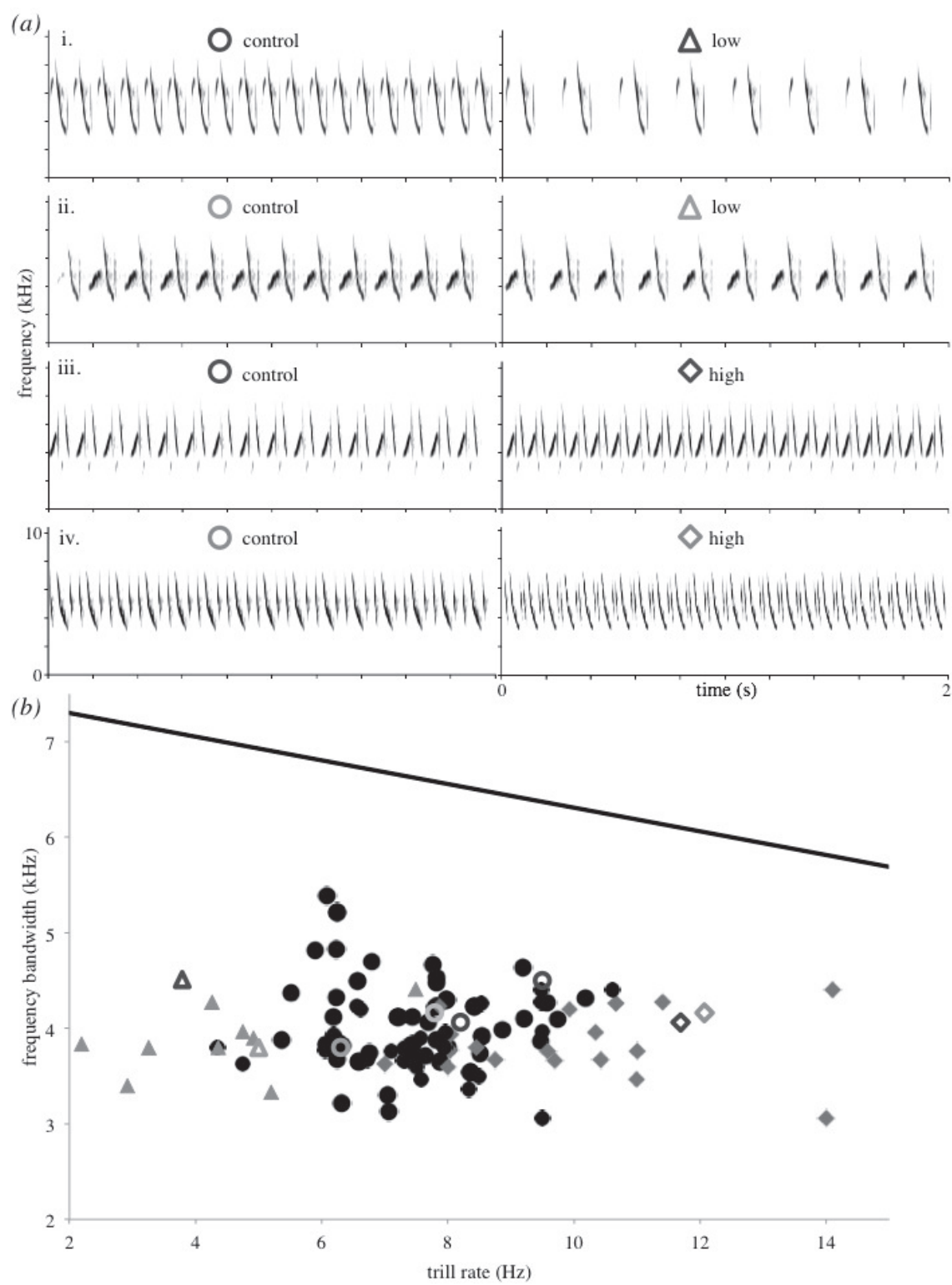


Figure 1. 2 Box and whisker plots of responses to playback of control- and either low-performance stimuli (Experiment 1) or high-performance stimuli (Experiment 2). Box boundaries indicate 25th and 75th percentiles, lines within the boxes indicate medians, whiskers above and below boxes indicate 10th and 90th percentiles, and circles indicate outlying points. Six response variables were measured for both experiments: a) broadcast song rate, b) flight rate, c) latency to first song, d) soft song rate, e) passes within 2 m of the speaker, and f) closest approach to the speaker. Note that for two response variables (c & f), smaller values represents more aggressive responses.

Experiment 1: All response variables show a pattern of more aggressive response to control- (gray) than to low-performance stimuli (blue), with a statistically significant difference by condition detected for d) soft song rate (Wilcoxon signed rank test  $n = 11$ ,  $Z = 2.72$ ,  $p = 0.004$ ). When reducing the six response variables in Exp. 1 to one PC variable, g) PC1 (lower left), the control- and low-performance trials differed significantly (Wilcoxon signed rank test  $n = 11$ ,  $Z = -2.66$ ,  $p = 0.008$ ).

Experiment 2: There were no statistically significant differences between control (gray) and high performance (red) with all males pooled, for all raw and PC response variables (gray and (Wilcoxon signed rank test  $n = 19$ , all  $p > 0.1$ ).

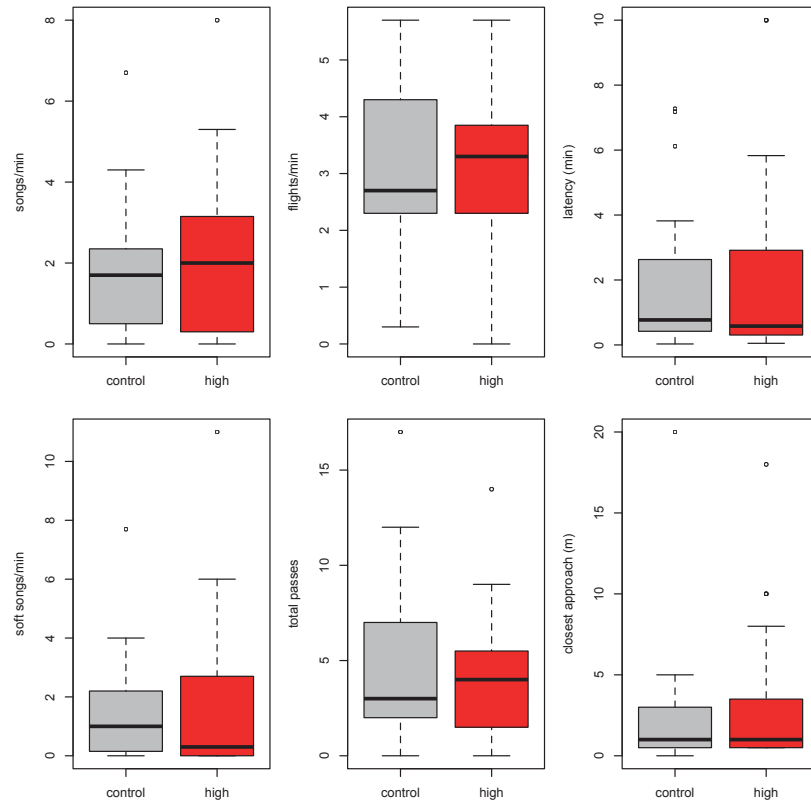
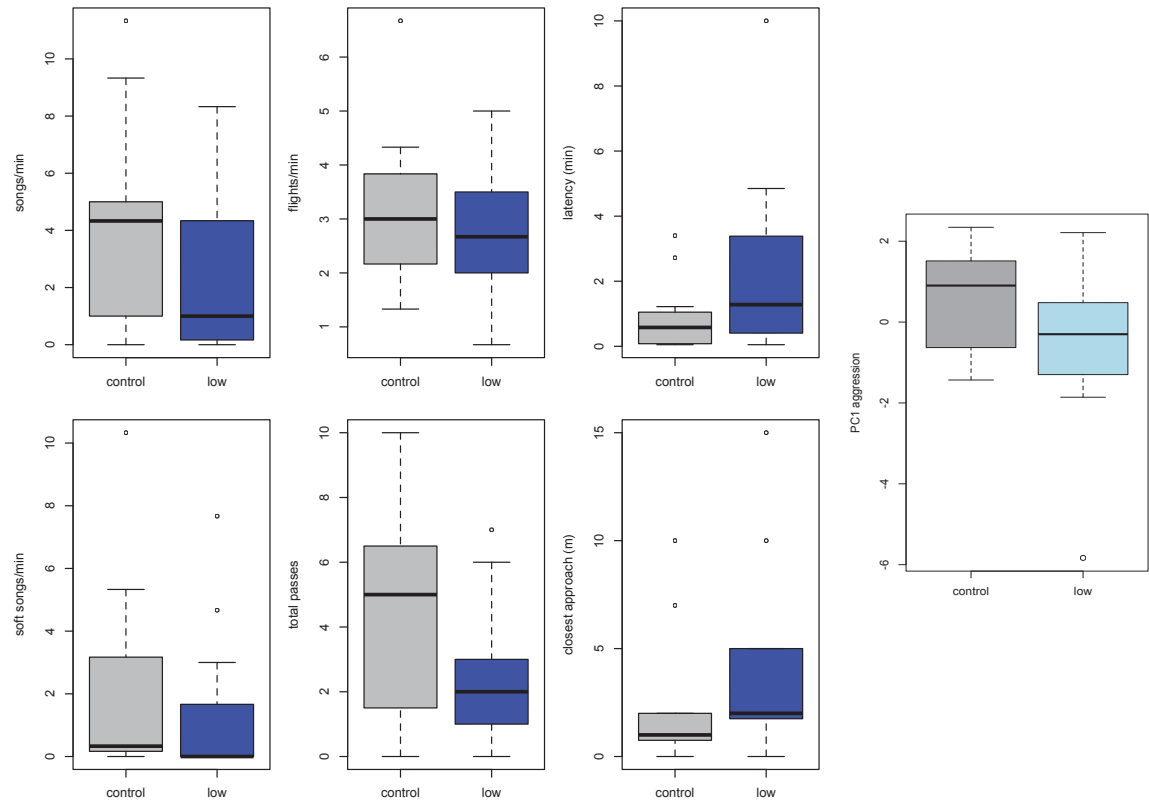
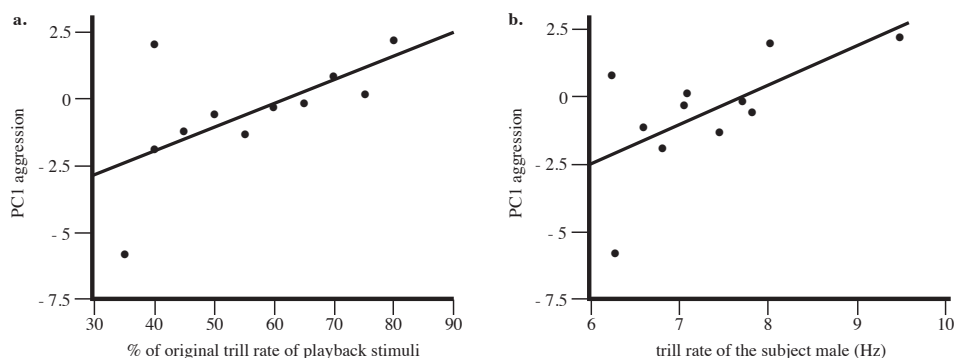




Figure 1.3 Playback responses as a function of signal vocal performance and subject vocal performance. Below relationships were identified as significant in the multiple regression analyses. **Experiment 1** (a,b): The Y axis represents the aggressive response to the low-performance trial (PC1 scores of six response variables reduced). Panel a: The X axis represents the percent of the original rate for the low-performance playback stimuli (with stimuli closer to the original rate having percentages closer to 100%, and stimuli with comparatively lower trill rates at lower percentages). Males were more aggressive to stimuli at higher performance levels that more closely resembled the original trill rate. Panel b: The X axis represents the subject male's trill rate. Males with higher trill rates (Hz) were more aggressive in response to playback (Table 1.2). **Experiment 2** (c,d): The Y axis represents the level of aggressive response differences (PC3 approach and proximity, Table 1), with positive values indicating a more aggressive response to high-performance over control-performance stimuli, and negative values indicating more avoidance of high-performance stimuli and thus more aggression to control-performance stimuli. Panel c: The X axis represents percent of the original rate for the high-performance stimuli (increases from the original rate of 100%, to 115-155% for high performance, Tables 1.5, 1.1, 1.4). The greater the vocal performance of the playback stimulus was increased, the less likely males were to approach aggressively in terms of distance and time spent close to the speaker. Panel d: the X axis represents the vocal performance of the subject males, with higher values indicating males with higher vocal performance (Table 1.5). Males with higher vocal performance themselves approached the high-performance speaker more aggressively.

#### Experiment 1



#### Experiment 2

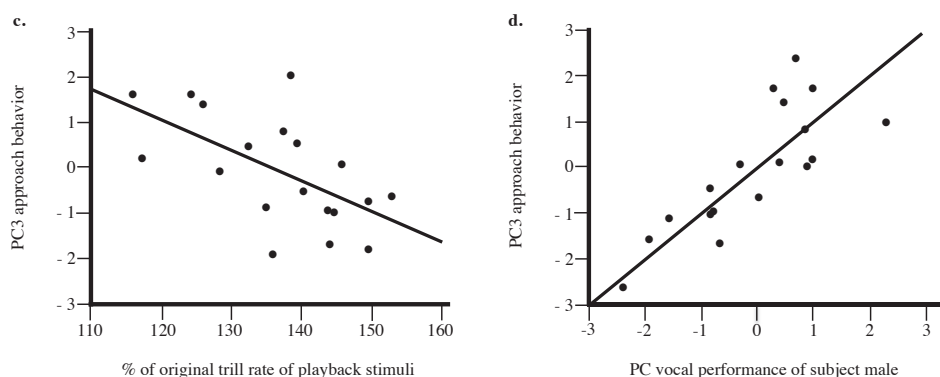
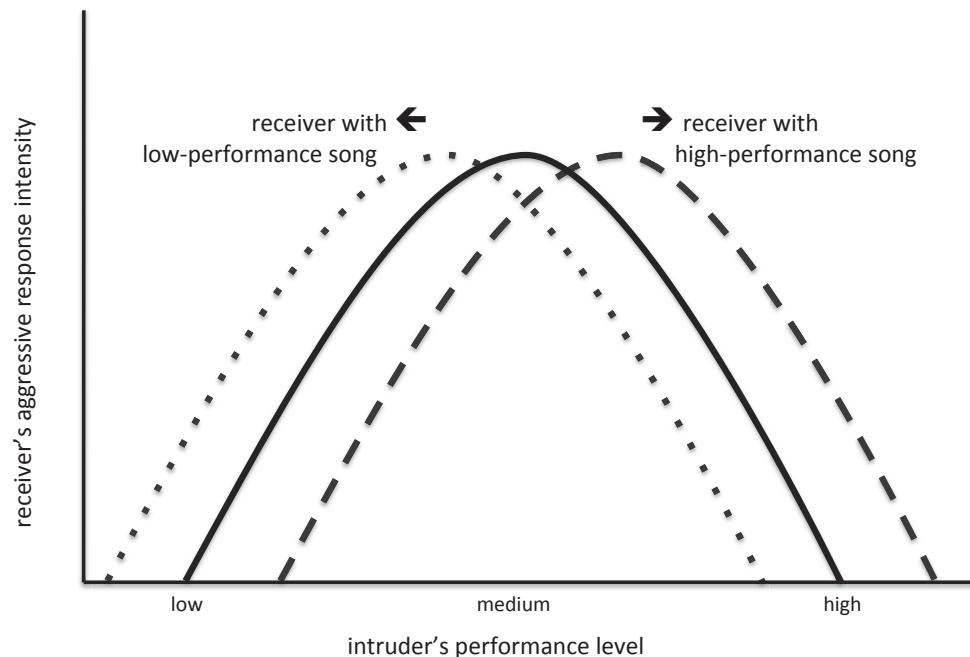


Figure 1. 4 A conceptual model describing the intensity of responses by territorial males to simulated territorial intrusion by intruders of various vocal performance levels. I expect response to vary by two factors: the level of threat posed by an intruder, and subjects' own vocal performances. The X axis represents the level of threat signaled by the intruder as the level of vocal performance, and the Y axis represents the aggressive response of the receiver (the territorial male). Response intensity has recently been modeled as a parabolic function, to account for the expectation that territorial males should avoid rather than confront intruders with high threat signals (solid line from de Kort et al. 2009a, modified from Collins 2006). I extend this model by accounting for expected variation in the quality of territorial subjects, here reflected in the vocal performance of the songs of playback subjects. As illustrated in this model, I predict that the parabolic function would shift to the right for territory owners producing high-performance signals (dashed line), and to the left for territory owners producing low-performance signals (dotted line). According to this model, as intruder signals increase in performance (levels of threat), low-performance territory holders would switch from aggression to avoidance sooner than would high-performance territory holders. These expectations are consistent with the outcomes of the multiple regression models and the relationships illustrated in Figure 1.3.



## CHAPTER 2

### THE DEVELOPMENT OF VOCAL PERFORMANCE: THE EFFECTS OF DEVELOPMENTAL STRESS, SENSORIMOTOR LEARNING, AND AGE

#### Abstract

The question of whether animal signals reliably encode information about their bearers is pertinent to investigating how communication signals evolve. The developmental stress hypothesis posits that costs experienced during ontogeny will manifest in signals produced later in life. While developmental stress is known to affect neural attributes involved in song learning, an open area of investigation considers how conditions experienced during ontogeny affect motor performance. Measuring vocal performance, i.e. the ability to produce a difficult signal, is likely to be a fruitful way for receivers to assess individual quality, as executing high-performance signals involves costs and constraints. I hypothesized that developmental stress negatively affects vocal performance, particularly when stress is experienced during both the sensory and sensorimotor phases in the swamp sparrow (*Melospiza georgiana*). I raised swamp sparrows and trained them with a regime of high performance song models in order to give them a performance challenge during development. I raised these birds in two groups, control and developmental stress conditions; in the latter condition, stressed birds received 70% of the amount of food of control birds during the entire period of hand-feeding. After hand-rearing, supplemental food was restricted and non-edible seeds were mixed in to the seed dispenser both at a two-thirds to one ratio of stress to control. This manipulation continued through the birds' lives including the sensorimotor phase of song acquisition. Vocal development was recorded until the birds had crystallized song and then again in the birds' second year of life. Copies made by control birds had significantly higher copying accuracy, consistent with results

from a prior study. Additionally, stressed birds produced songs with significantly lower frequency bandwidth, and thus lower vocal performance. Testing all song types with all birds pooled, vocal performance significantly increased between years, with developmentally stressed birds producing songs that had significant increases in frequency bandwidth. These results illustrate previously undocumented negative effects of developmental stress on vocal performance. Variation in birds' natural environments during sensory and sensorimotor phases may lead to the variation in vocal performance observed in the wild. Thus for receivers, vocal performance may offer a window into assessing birds' condition early in life. Because vocal performance increased with age, I infer that vocal performance improves with increased practice and could also indicate survival ability.

### **Introduction**

The topic of honesty in animal communication is currently a major focus of research. Signal reliability appears to be maintained through mechanisms such as costs including both receiver dependent costs, such as retaliation, and receiver-independent costs, such as production, developmental, and maintenance costs (Maynard Smith and Harper 2003, Searcy and Nowicki 2005). An emerging concept that can also explain signal reliability is that of constraints. Animals are often limited in the signals they can produce, therefore an animal's quality or condition may be revealed by signaling performance. Performance can be defined as the ability to execute ecologically relevant tasks (Irschick et al. 2008). Tasks that are physically challenging, such as sprinting in lizards (Lailvaux and Irschick 2006), operate within specific physiological costs and constraints. Motor constraints thus limit the expression of difficult behaviors, and not all individuals may be able to achieve maximal performance. Performance is relevant to signaling behavior, particularly in regard to mating, because individuals that execute high-performance mating displays could

distinguish themselves from competitors, and thus enable assessment and discrimination by signal receivers (Byers et al. 2010). Prior research has demonstrated the importance of motor performance in adult behaviors, such as competition and mate choice, yet few studies have investigated how traits involving motor performance develop.

Knowledge of how traits are shaped through developmental processes leads to a better understanding of how evolution acts on such traits (West-Eberhard 2003). Songbird vocal communication has served as a key model system for behavioral development for over half a century (reviewed by Catchpole & Slater 2008). From the production side, songbirds learn their songs through memorization and practice (Marler & Peters 1977, 1982). Like human children, young birds listen to adult song models during a sensitive phase and commit those songs to memory during the memorization phase. Later birds go through a protracted phase of vocal practice, which is akin to the babbling of human children, in the sensorimotor phase. Studies on song development have demonstrated a link between ontogenetic processes involved in song acquisition and variation in song features that females use to assess males (Nowicki et al. 1998, 2002, Buchanan et al. 2003, 2004). More specifically, young birds face a variety of stressors, such as poor nutrition, during the period of song learning and brain development. Regions in the brain termed “song nuclei” are known to function in song memorization and production, and the size of these nuclei has been shown to correlate with the expression of song between sexes, the size of song repertoires, and learning ability both within and among species (DeVoogd et al. 1993, Szekely et al. 1996, Brenowitz et al. 1997, Airey et al. 2000, Nottebohm 2005). Studies have documented that when birds face early stress, stressed birds show reductions in two song nuclei, the RA and the HVC (e.g. Nowicki et al. 2002, Buchanan et al. 2004). Thus stress experienced during development appears to affect the growth of song nuclei in the brain with corresponding deficits in adult song production. The developmental stress hypothesis

posits that signalers' early condition can be indicated by adult song phenotypes because of the overlap of song learning, brain development, and the corresponding timing of early stress (Nowicki et al. 1998, Buchanan et al. 2003). Evidence for the effects of stress experienced during ontogeny on adult condition has also been shown in a variety of mammals including humans (e.g. rats Rots et al. 1996, humans: reviewed by Frodl and Keane 2013), with phenotypic effects from increased stress responses to abnormal development of the hypothalamic-pituitary-adrenal axis, but the connection to sexually selected traits remains little studied outside of birds.

In the last decade, evidence from numerous species has mounted in support of the developmental stress hypothesis, and a variety song features appear to be compromised by stress (reviewed in Podos et al. 2009, MacDougall-Shackleton & Spencer 2011, Spencer & MacDougall-Shackleton 2012), but with some mixed results (see Naguib and Gil 2005, Gil et al. 2006, Zann and Cash 2008). The song features affected by developmental stress seem to vary by the timing and type of stress implemented, but a commonality of these studies is that birds were stressed during the memorization phase of song learning. In an early study, Spencer et al. (2003) found that nestling zebra finches (*Taeniopygia guttata*) exposed to either elevated stress hormone levels (corticosterone) or nutritional stress showed significantly reduced song phrase duration, song complexity, and peak frequencies compared to control birds. Stressed male European starlings (*Sturnus vulgaris*) showed reduced song output, shorter song bouts, and reduced repertoire sizes as adults compared to unstressed males (Buchanan et al. 2003, Spencer et al. 2004). However, a few studies which varied the size of broods within the natural range for zebra finches following the logic that larger broods would face more nutritional stress than smaller broods, found no detectable difference between groups in song quality (Gil et al. 2005, Naguib and Gil 2005), and subsequently found no differences in female preferences between songs of males in

stressed and unstressed conditions (Naguib et al. 2008). In contrast, other studies with similar brood-size manipulations did detect effects on song complexity, consistency, and phrase length in zebra and Bengalese finches (Spencer 2003, Soma et al. 2006, Holveck et al. 2008). We do not yet know if the song features shown to be affected by developmental stress are features that also face motor constraints in their production. Although a great deal of empirical work has shown support for this hypothesis, questions remain regarding the effect of developmental stress on song production and its evolutionary consequences.

A largely unexplored area of the developmental stress hypothesis concerns how developmental conditions shape the expression of vocal performance, i.e. the ability to coordinate multiple motor systems adeptly during song production, especially during the production of challenging vocal sequences. Unlike learning parameters such as imitative accuracy and complexity, which for many species appear to be encoded for life, vocal performance may either reflect a bird's condition during the early period of song acquisition or its current condition. Recent studies provide some hints that performance may also be affected by developmental stress. For example, stressed birds have been shown to have lower song output, reduced note or song duration, or lower consistency (Buchanan et al., 2003, Dreiss et al., 2008, Gorissen et al., 2005, Holveck et al., 2008, Zann and Cash, 2008), although whether these song features operate under performance limits is not known for these study species. The conceptual interface between vocal performance and song learning is detailed in a recent review (Podos et al. 2009). One general expectation that emerged posits that developmental stress may affect vocal performance particularly if stress is experienced during the sensorimotor phase of song practice. The sensorimotor phase is crucial as it is the first time that birds begin to transform memories to vocal output and to train the vocal tract to attempt the dynamic modulations of the syrinx necessary for executing the frequency and timing of species-typical song (Suthers and Zollinger 2004,

Suthers et al. 2012). It is an open question as to whether early stressors affect vocal performance as they appear to affect other aspects of song production such as copying accuracy, repertoire size, and neuromorphology (Nowicki et al. 2002, Spencer and MacDougall-Shackleton 2011).

Vocal performance describes aspects of song that are difficult to produce and often encounter performance limits. In a family-wide comparison, emberizid sparrows were found to face a trade-off between high trill rates and wide frequency bandwidths in singing (Podos 1997). From this analysis an upper-bound emerged, which describes a limit in how these two features are maximized (Fig. 2.1c). Indeed this trade off has emerged in over forty species of songbirds (Passeri; reviewed in Podos et al. 2009), South American woodcreepers (birds which do not learn their songs, Dendrocoloptinae; Derryberry et al. 2012), one species of seabird (Stercorariidae; Janicke et al. 2008), and neotropical singing mice (Cricetidae; Pasch et al. 2011). Using this trade-off, one can then plot the trill rate and frequency bandwidth for an individual sparrow's song, and measure the distance to the Emberizid upper limit; this metric has been referred to as the vocal deviation (Fig. 2.1c; Podos 2001, Ballentine et al. 2004). Variation among male songbirds in their quality is likely to be revealed by their vocal deviations, i.e. how well they maximize trill rate and frequency bandwidth relative to population levels (Nowicki et al. 1998, Ballentine et al. 2004, Podos & Nowicki 2004, Dubois et al. 2011, Moseley et al. 2013).

One model species that has been the subject of empirical studies on the topics of song learning, developmental stress, and vocal performance is the swamp sparrow (*Melospiza georgiana*). In this species the process of male song learning is particularly well understood. Swamp sparrows memorize song models that are presented at 90 days of age, and then practice these song models during the sensorimotor phase in their first spring (Marler & Peters 1977, 1982). In the late spring and early summer, swamp sparrows



crystallize songs in which syllables are repeated in rapid succession, forming “trills” (Fig. 2.1a). Prior studies by Podos and colleagues indicate that trills vary in how difficult they are to produce, and specifically that songs with fast trill rates and wide frequency bandwidths require greater levels of vocal proficiency (Podos 1996, 1997, 2001, Podos and Nowicki 2004, Podos et al. 2004). Young swamp sparrow males tutored with song models with artificially increased trills (Fig. 2.1b) proved able to memorize these models, but encountered motor challenges in their reproduction (Podos 1996, Podos et al. 1999, 2004). In another study, tutoring swamp sparrows with both lowered and elevated performance songs, young males copied songs of normal to high performance levels more accurately, while increasing the trill rates of slow tutor models (Lahti et al. 2011). It appears that young birds adjust performance levels to their own motor capabilities from both high- and low-performance models (Lahti et al. 2011).

A previous study of developmental stress in swamp sparrows demonstrated that males who experienced early nutritional stress showed deficits in learning, specifically stressed males produced songs that were poorer copies of tutor models than did control males (Nowicki et al. 2002). We recently proposed that if the effects of stress extend into the sensorimotor period, additional negative impacts on song ontogeny might be revealed in vocal performance (Podos et al. 2009). Birds experiencing stress during the sensorimotor phase may lack abilities to reproduce challenging vocal features such as rapid trills or broad bandwidth notes, fail to achieve high vocal performance in practice, and thus crystallize songs with low performance levels. Ultimately, according to this hypothesis, adult songs would thus indicate not only bird’s neural capacities as they existed during memorization, but also the motor-performance abilities achieved during sensorimotor learning.

I address the topic of how vocal performance may be affected by costs, specifically nutritional stress, experienced throughout the period of sensory and sensorimotor learning

in swamp sparrows. To date, tests of the developmental stress hypothesis have focused on manipulations during the first few months of life, but in the present study, I extend this manipulation through the sensorimotor phase. I predict that males experiencing stress throughout the entire process of song acquisition will crystallize songs with lower vocal performance. Alternatively, if vocal performance is a signal of current condition, adult vocal performance may not be affected by early stress, or performance capabilities may be improved upon when body condition is higher and when a bird is further removed from experiencing early stress. I thus assess how early costs affect vocal performance capacities over two time frames – the first year of life and the second breeding season (when birds are two years old). Previous studies have focused on measuring song output in birds' first year spring and summer. In fact, with each successive breeding season, male birds experience a jump in testosterone levels and a coinciding re-growth of the song nuclei regions in the brain (Brenowitz et al. 1991, Nelson 2000, Meitzen et al. 2009). A previous study on wild swamp sparrow males by Ballentine (2009) suggested that vocal performance increases with age, as older birds in this study produced songs with higher vocal performance. Therefore birds may have the opportunity to increase their realized performance through practice in the second year, and thus performance may be a more accurate signal of age. If males that experienced early developmental stress show improvements in their vocal performance later in life, then this would suggest vocal performance may be tied to current condition.

## **Methods**

### **Study animals and housing**

I collected swamp sparrow nestlings of four to eight days post-hatching from nests in a population at the Quabbin Reservoir in Franklin County in western Massachusetts, in May-July 2009. Nestlings' experiences up to this age are unlikely to have any lasting effects

on the development of their learned songs, especially given that, like other songbirds, swamp sparrows are altricial and hatch with their sensory and neural structures in only rudimentary form. Indeed male nestlings do not appear to learn songs presented to them during their first days in the nest (Kroodsma 1978), nor do they crystallize songs presented in the first 30 days of life (Marler and Peters 1987). The sex of each nestling was identified using a genetic test of a blood sample (Avian Biotech, Tallahassee FL), and males and females were separated into two large, walk-in acoustic chambers and placed in individual cages (194 x 194 x 207 cm) (Podos et al. 1999, 2004). Twenty-one males were collected, and nestlings were split into two groups (control or stress) using a coin flip for the first nestling and then a balanced design for each of the following nestlings within each nest, such that siblings were divided as evenly as possible. In order to keep the nestlings warm, groups of individuals were put into nests of similarly aged individuals. Nestlings were hand-reared in sterilized swamp sparrow nests until fledging, then in groups of 5-8 in common cages (84 x 74 x 44 cm) to independence, and finally housed in individual cages (46 x 22 x 26 cm). Birds were kept at the natural light:dark schedule throughout the study, and had access to perches, biweekly baths, and ad libitum water. Nestlings and fledglings were fed a blended mixture of commercial turkey feed, carrots, eggs, ground beef, calcium, and vitamin powder. Control birds were fed until sated, and the volume fed was measured to the nearest 0.05mL (Nowicki et al 1998, 2002). Developmentally stressed birds were then fed 70% of the amount their control-siblings had eaten that hour. As the birds grew to independence, this diet gradually transitioned to include a dry food supplement and mealworms, which were also given in a ratio of 100 control: 70 stressed. At roughly 21 days of age birds had ad libitum access to a commercial finch seed mixture supplemented with egg food. In order to produce a continued foraging challenge for the stressed group, I mixed in large black-oil sunflower seeds at roughly 30% of the seed mix. Swamp sparrows cannot crack these seeds

and thus had to pick through them for the seeds they could process. I measured mass to the nearest 0.1 g daily from age 4-90 and every other day from day 60-120. Males were kept in captivity for 48-51 months for this study. Six males did not survive to adulthood (4 control males and 3 stress males), resulting in a sample size of 14 males, seven in each group. All protocols were approved by the University of Massachusetts Institutional Animal Care and Use Committee (protocol #28-10-02).

### **Song training regimes**

Tutor models were constructed from ten swamp sparrow songs recorded from the local Quabbin population (Franklin County, MA) using a Sennheizer directional microphone and Marantz digital recorder. Swamp sparrow songs are approximately two seconds in duration and are comprised of a repeated syllable that contains between two and five notes. Songs used to construct training models naturally ranged in trill rate between 6.2-10.6 Hz (Table 1). Using Signal Software (Engineering Design 2003), I constructed one regime of ten training songs for all birds (Fig. 2.3), as done in previous studies in order to insure a diversity of acceptable training models (Podos 1996). I included two control songs which remained at the original, wild-recorded trill rate, and the remaining eight songs in each regime I experimentally modified to challenge birds' vocal performance capacities. Trill rates were adjusted by deleting silent intervals between notes and syllables proportionally, thus preserving the original structure of individual notes within syllables (Podos et al. 1996, 1999). These syllables were increased to 115 - 155% of their original rates, resulting in trill rates of 7.6 - 16.5 Hz. The original (control) and modified syllables were then concatenated into 2 sec-long songs. Songs were played at the natural rate of one song every 10 seconds. Each tutor model song type was played for six minutes, twice a day, and presented in a random order each morning and evening. Song training was conducted for two hours a day – one hour between 7am and one hour at 5pm. Training began at 9-29 days of age and

continued until 118-148 days of age; this time period encompasses the sensitive period for song acquisition in this species (Marler & Peters 1988).

### **Analysis of learned songs**

From April through late June, I recorded the vocalizations of each bird for four hours in the morning, two days each week, rotating birds through eight individual recording chambers. Recordings thus started during subsong and continued until birds had crystallised their song type repertoires. Upon reviewing the daily recordings I identified all crystallised song types in order to determine each bird's full repertoire of song copies. For each copy by an individual bird, three renditions were chosen from the last three days of recording for quantitative analysis.

The goals of the analyses were to compare the copying accuracy of song copies to tutor models and vocal performance for the control and developmental stress groups. The copies were assigned to specific tutor models by visual inspection (DLM and JP independently) of note structure and sequences within syllables from spectrograms (as in Podos 1996, Podos et al. 1999, Lahti et al. 2011). Some songs were not easily identifiable to a tutor model, for these the best-match was determined by spectrographic cross-correlation analysis (SPCC) using SIGNAL (Engineering Design Belmont, MA). First, I observed and documented qualitatively the syntax and note composition of each copy. In particular, I documented note omissions (in which males omitted notes from their copy that were contained in the tutor model, and the occurrence of broken syntax, gaps in the normal trill sequence (Podos et al. 1999). Second, I assessed the accuracy of note and syllable reproduction separately, using SPCC, a method which assesses the similarity between model songs and their copies in terms of frequency and amplitude structure over time. I performed this analysis both whole syllables as well as individual notes within the syllable for each copy. Cross-correlation scores generally match designations of song similarity

based on visual examination of spectrograms (Podos et al. 1999). SPCC calculations were performed both with and without normalizing for frequency offset, on spectrograms constructed with 128-point fast Fourier transformations (FFTs) and 100 time steps, within a frequency range of 1.5 to 10.5 kHz. The results from the two methods were similar and I report only the values without frequency-shifting to be conservative. SPCC scores were compared between groups using a Wilcoxon sign-ranks test.

The vocal performance of subjects' songs was assessed by first measuring the trill rate and frequency bandwidth for the three renditions of each crystallized song. Trill rate was determined by measuring the syllable length across the eight central syllables of each song (or fewer if a song contained fewer than eight syllables), and then taking the inverse of this value to generate syllables per second (Hz). Frequency bandwidth was determined by measuring the minimum and maximum frequency at a threshold of 24 dB from the peak frequency (Podos 1997). Then average values were graphed on a plot of frequency bandwidth by trill rate, and the distance of each point was measured to the upper-bound line for all Emberizids (Podos 1997). In this way the vocal deviation, or distance from the upper-bound, was determined for each song copy. A greater distance from the line indicates low performance, and a smaller deviation indicates high performance. We then compared the average trill rate of each copy to that of its model. Finally vocal deviation was compared across nestlings and groups.

In year two of this experiment, I tested if males were able to recover or increase their realized vocal performance through practice in their second spring. The foraging challenge of including sunflower seeds mixed with regular seed was continued for the developmental stress group. I used the birds' first-year trill rate, frequency bandwidth, and vocal deviation (VocDev) as a baseline and compared the VocDev of the birds' songs in the second breeding season to this value ( $\text{VocDev}_{2\text{nd yr}} - \text{VocDev}_{1\text{st yr}}$ ). The resultant difference in

VocDev for birds stressed and non-stressed in the second breeding season was compared using a Wilcoxon signed-ranks matched-pairs test.

For all the above tests I performed analyses on both a per song and per bird basis. Twelve of the 14 males possessed more than one song type (2-4) in their repertoires. For each male, I took the average of each parameter (copying accuracy, trill rate, frequency bandwidth, vocal deviation) across his song types, and conducted analyses on the averages. I report these analyses below and identify analyses on the per individual basis with N's for number of males, or on the per song basis with N's for the number of songs.

## **Results**

### **Patterns of Song Learning**

The fourteen males produced 34 crystallized vocalizations which they sung repeatedly in a stereotyped manner in both years. Males produced one to four song types each, but these song types ranged from songs identifiable as copies of tutor models by eye and/or SPCC ( $N_{\text{songs}} = 18$ , for examples see Fig. 2.4) to songs composed only of one note per syllable in a trill ( $N_{\text{songs}} = 16$ , for examples see Fig. 2.5). Henceforth I will refer to these two kinds of songs as “copies” and “single-note trills.” These single-note trills may be copies with the omission of one or more notes per syllable, especially since note omissions are known to occur when copying high-performance tutor models, or they may be examples of improvisations (Podos 1996, Nowicki et al. 2001) or merely the innate species specific pattern of vocalizations (Marler and Peters 1981). Five males in the control group produced copies, while four males in the stress group produced copies.

Males' vocalizations were reproduced with similar modifications as previously observed (Podos 1996, 2001, 2004, Lahti et al. 2011). First, in some cases, songs were produced at trill rates slower than their tutor models. Second, in other cases, between 1 and

3 notes were omitted, and were thus produced at trill rates more similar to the tutor model (Fig. 2.4d,e). Third, some songs, including single-note trills, were produced with broken syntax, i.e. silent gaps in the normally continuous trill. Broken syntax was often observed in songs exhibiting extreme trill rates (Fig. 2.5 middle row), but not in copies of tutor models presented at control rates. Fourth, in a new finding, some songs were shortened well below the typical two-second duration of the species.

Both copies and single-note trills were distributed across the performance range of tutor models presented (Fig. 2.6a). Thus males in both control and developmental conditions appeared to copy models across the range of performance levels.

### **Copying Accuracy**

#### **Copies**

Copies differed by group in copying accuracy in the expected direction. Specifically control males copied syllables of their tutor models better than did developmental stress males, as seen in the degree to which copy syllables matched model syllables in SPCC (Wilcoxon signed-ranks test,  $N=18$  copies, Fig. 2.7,  $W=62$ , one-sided  $P = 0.018$ ,  $\bar{x}_{\text{control}} = 0.84$ ,  $\bar{x}_{\text{stress}} = 0.69$ ,  $\beta = 0.15$ ;  $N=9$  males,  $W=18$ ,  $P = 0.03$ ,  $\bar{x}_{\text{control}} = 0.84$ ,  $\bar{x}_{\text{stress}} = 0.65$ ).

#### **Single-note Trills**

Single-note trills varied in the structure and acoustic properties of the note repeated. For each trill, the note repeated was determined to match a tutor model note by its maximum SPCC score for similarity. Notes in trills matched their putative tutor model notes by 0.76 for the best match (range of best match: 0.53-0.86; 1.00 being a perfect match). The average similarity between a trill note and any of the possible model notes was 0.23 (range of average 0.12-0.51).



## Vocal Performance

### Differences Between Treatment Groups

In 2010, the first year in which males produced crystallized songs, the songs of control and of developmental stress males differed in their vocal performance. When comparing all songs, copies and signal-note trills, the difference between groups in vocal deviation was also significant with control males having higher vocal performance (lower deviation) than stressed males ( $N = 14$  males,  $W=10$ ,  $P=0.036$ ). This same pattern was also significant when comparing copies alone or single-note trills alone ( $P < 0.05$ ). This difference was particularly apparent in frequency bandwidth (Wilcoxon signed rank test,  $N = 14$  males,  $W = 45$ ,  $P = 0.004$ ;  $\beta_{\text{control-stress}} = 0.358$  kHz) with control males having higher bandwidths (Fig. 2.8), but there was not a statistically significant difference between the groups in trill rate ( $P > 0.1$ ). Frequency bandwidth significantly differed between groups when testing all songs together, copies alone, or single-note trills alone ( $P < 0.04$ ).

### Differences Between Years

Between 2010-2011, birds improved their vocal performance of learned songs (both copies and single-note trills, some examples illustrated in Fig. 2.9), that is vocal deviation was significantly smaller in the second year than in the first (Fig. 2.10; Wilcoxon matched-pairs signed-ranks test,  $N = 14$  males  $W=92$ ,  $P = 0.0054$ ,  $N = 34$  songs,  $\beta_{\text{VocDev:2011-2010}} = -0.23$ ,  $V = 401$ ,  $P = 0.0153$ ). With all males pooled, significant differences were also seen in trill rate and frequency bandwidth ( $\beta_{\text{Trill Rate}} = 0.29$  Hz,  $\beta_{\text{Bandwidth}} = 0.197$  kHz,  $P < 0.05$ ). Looking at the groups individually, control males significantly increased trill rate (Fig. 2.11;  $V = 30$ ,  $P = 0.013$ ), while stressed males significantly increased frequency bandwidth ( $V = 11$ ,  $P < 0.001$ ). Both groups showed increases in the other parameter, but not at statistically significant levels. Together the significant increase in one parameter coupled with the slight but not statistically significant increase in the other parameter, resulted a significant

decrease in vocal deviation across years for each group when analyzed separately (control:  $V = 126.5$ ,  $P = 0.0096$ , stress:  $V = 134.5$ ,  $P = 0.0032$ ). Despite the marked increase in frequency bandwidth and decrease in vocal deviation within the stress group, stressed males still did not completely recover vocal performance to the level of control males in their second year. Control and stress groups differed in vocal deviation in 2011 for copies analyzed alone ( $N = 9$ ,  $W = 2$ ,  $P = 0.032$ ), and groups showed a trend for all songs together ( $N = 14$ ,  $W = 12$ ,  $P = 0.064$ ).

## **Discussion**

The main goal of this study was to test, whether developmental stress results in compromised vocal performance capacities. My results demonstrate that a brief period of severe nutritional stress and a prolonged foraging challenge with limited supplemental food can indeed have significant effects on song development, not only on the accuracy of how song is copied, but also on the performance levels at which song is produced.

The first learning trait I considered was copying accuracy. As in Nowicki et al. (2002), songs learned by control male swamp sparrows showed significantly higher copying accuracy than did copies produced by developmentally stressed males. Nowicki and colleagues (2002) further demonstrated that male swamp sparrows raised under early nutritional stress had significantly smaller brain regions of the song learning neural circuit. Specifically, the volume of both HVC and RA were reduced as compared to control males. These regions are known to be involved in song memory and production, specifying the timing and fine structure of note patterning (Vu et al. 1994, Yu and Margoliash 1996, Prather et al. 2011). Together the neural and behavioral deficits suggest that developmentally stressed birds are compromised neurologically and that this affects accuracy in song learning of both normal- and high- performance songs. When tested in the

lab, wild-caught adult female swamp sparrows have been shown to give more copulation solicitation displays in response to songs that more closely matched tutor models than to songs poorly copied or with introduced innovations in trill structure, such as broken syntax (Nowicki et al. 2002b). Adult female swamp sparrows tested in the lab have also displayed more to higher levels of vocal performance of song variations found in nature (Ballentine et al. 2004), and to songs of artificially increased in performance (Moseley *unpub. data, see Chapter 3*). Females thus appear to attend to the both song copying accuracy and song performance level. Therefore songs of control males in the present study would be likely to function better in mate attraction than the more poorly copied songs of stressed males.

I next considered the possible influence of developmental stress on song performance features. My manipulation continued once males reached independence and involved a reduction in supplemental food (mealworms and eggfood) and a foraging challenge of uncrackable seeds mixed in the seed dispenser for stressed males. In this way males were also stressed during their sensorimotor phase, although they did have free access to *ad libitum* edible seeds. My results show that developmentally stressed males did not achieve as high frequency bandwidths as did control males, which resulted in significantly higher vocal deviation values (i.e. lower vocal performance) for stressed males. The negative effects on frequency bandwidth were evident regardless of whether or not the song was a complex, multi-note song or a single-note trill. Therefore two aspects of song quality – accuracy and performance – were both affected by developmental stress in the direction predicted, thus providing evidence for mechanisms that could enable performance to act as an indicator of early condition. Whether the manipulation during the sensorimotor phase additionally led to decreases in vocal performance among stressed birds or whether the early stress was sufficient to lead to low vocal performance is unclear. Deficiencies in frequency bandwidth experienced by stressed birds may in theory be attributed to the

sensory phase manipulation, to the sensorimotor phase manipulation, or to some combination of the two. Both sets of stresses may have affected the cognitive and perceptual abilities of males and their vocal practice abilities later in the spring. Yet some lines of evidence suggest that sensorimotor phase constraints provide a more likely explanation for the observed pattern (Schmidt et al. 2013). Young birds likely face challenges in coordinating multiple motor systems to produce such large changes in frequency at rapid rates. Indeed practice with the vocal apparatus seems necessary to achieve vocal complexity. In a study of young cardinals (*Cardinalis cardinalis*) in which respiration intake and expiration was measured, birds during the sub-song phase were unable to precisely coordinate expiratory breathing with syringeal activation (Suthers 2004). Additionally, juvenile song sparrows (*Melospiza melodia*) introduced vocal tract modulations when songs were nearly crystallized (Podos et al. 1995). Putting these concepts together, the sensorimotor phase may be a crucial stage of vocal performance development because of the interactions between vocal memory and vocal output as birds matching neural memories of model songs to their motor capabilities during the first attempts at song production. It has been suggested that the protracted period of memorization and practice can be explained as an adaptation to produce better vocal output (Podos et al. 2009).

Males generally seemed to place a premium on reproducing the high trill rates of model songs, as 12 of 14 males produced rapid trills consisting of only a single note per syllable. Even individuals who were able to reproduce model songs with high copying accuracy also produced single-note trill song types in their repertoire. When comparing only single-note trills between conditions, control birds maintained significantly higher bandwidths than did stressed birds. Explanations for these findings include the possibility that the fine syllable and note structure of high-performance models are particularly difficult for stressed birds to recognize and memorize. Alternatively, young birds may

successfully memorize rapid model song structure, but face morphological limits in reproducing fast trills without omitting notes within syllables as shown by Podos and colleagues (1996, 1999, 2004a,b). Thus stress may shape how the vocal tract develops during sensorimotor learning. If achieving high vocal performance takes precedence over copying accuracy, then birds might be likely to drop notes or make other modifications in order to signal high performance (e.g. Podos 1996, Lahti et al. 2011). By producing high performance single-note trills, stressed birds may mask other deficiencies if vocal deviation is the most salient feature to receivers.

Another main result in this study is that the vocal performance of subject males in both conditions increased the first year to the second. Control and stressed groups, however, increased their vocal performance in different ways: control birds significantly increased their trill rates, while stressed birds significantly increased their frequency bandwidth. When males were two years old, stressed males still produced learned song copies with significantly lower frequency bandwidth than did control males. These results suggest a mechanism that could explain observations of changes in vocal performance in the wild. In a study of a wild population of swamp sparrows, Ballentine (2009) found that older males produce higher performance songs than do younger males. This study suggested two potential mechanisms, which may operate concurrently: first, that males with higher vocal performance survive better, and second that males might increase their vocal performance as they age. By tracking individual males over time, the present study supports the latter hypothesis; as males age and continue to practice song, they might further train their vocal apparatus to achieve higher vocal performance. It is not yet known if males with higher vocal performance also have increased survivability. In another study that bears upon this hypothesis, DuBois et al. (2009) found that wild territorial males slightly increase their vocal performance between neutral and aggressive contexts,

exhibiting lower vocal deviation in response to conspecific playback. Comparing the effect sizes of performance modulation between the previous study of wild males between contexts and the present study of lab-reared males, there is a greater difference in vocal performance between years than between aggressive and neutral contexts (change in vocal deviation between contexts = -0.11, between years = -0.23). If vocal performance is a reliable signal of male quality, then the degree to which males are able to increase their vocal performance in aggressive situations should be minimal, or at least should scale similarly across individuals. It is thus logical that a change in vocal performance with age should be much greater than a change due to the nature of a momentary aggressive encounter, the former potentially indicating the effect of increased practice and skill. Receivers, whether rival males or potential female mates, would benefit from signals indicating the signal bearer is older and therefore likely a better competitor or mate.

Song features of other species have been shown to increase in signal magnitude with age, and two such examples are repertoire size in nightingales, *Luscinia megarhynchos* (Kiefer et al. 2006) and trill consistency (a potential performance characteristic) in banded wrens *Thryothorus pleurostictus* (de Kort et al. 2009). In the latter study, banded wrens were marked as nestlings and their songs were recorded when they were adults at one, two, and sometimes three years of age. Males showed increases in the consistency of their trilled songs between one- or two-year time frames, and other males responded differently to playback of younger versus older males (de Kort et al. 2009). Age has been demonstrated to correlate with social dominance in a number of species of birds, leading to better access to food and territories (e.g. Smith 1984, Arcese and Smith 1985, Piper and Wiley 1989, Sandell and Smith 1991). In a study of blue tits (*Parus caeruleus*), extra-pair males were older than the social males they cuckolded (Kempnaers et al. 1997). There is also evidence in other taxa that signal values and mating success can increase with age, for example older red deer

(*Cervus elaphus*) have more points and longer antlers (Mitchell et al. 1977), and older bighorn rams (*Ovis canadensis*) have longer horns and tend to sire more offspring (Coltman et al. 2002). Therefore various receivers may benefit from signals that provide reliable information about the age of signalers, whether they are competitors or potential mates.

In conclusion, vocal performance appears to be an indicator of both early condition and age. This study has demonstrated a mechanism by which vocal performance may be a reliable signal of male quality as it pertains to viability and to the rearing environment through the first migration and early spring. Unpacking the exact timing and mechanism of nutritional stress – whether deficits were because of musculature or neural physiology – will lead to a better understanding of how vocal performance acquired as a signal. As males experience variable foraging environments in which they may experience poor nutrition, not every individual is likely to reach its full potential of vocal performance. If young males experiencing poor early nutrition do so because of poor parenting by fathers, and if low paternal feeding is inherited, then poor song quality may be indicative of parental investment. Females assessing male song would benefit from information indicating the direct or indirect benefits provided by a mate. In this scenario, females may gain direct benefits from high-performance males if they engage in more paternal feeding of young. If nutrition during the sensorimotor phase is key to practicing frequency modulations, then high vocal performance may also indicate successful migration and foraging upon return. Additionally, males with higher vocal performance appear to deter rival males from engaging in aggressive disputes (de Kort et al 2009b, Moseley et al. 2013), thus females may gain direct benefits from pairing with high-performance males. Future studies could test these questions pertaining to the timing of stress and measure any cross-generational effects of nutrition and vocal performance. Such tests would provide further insight into the mechanisms that maintain signal reliability through costs or constraints.

Table 2. 1 Details of performance modifications made to the ten tutor model stimuli. These songs varied in the notes that comprised the repeated syllables. Naturally-occurring songs were modified in trill rate to varying degrees, which resulted in increased vocal performance levels and thus lower vocal deviations. Syllable type refers to the note type and order of notes within a syllable as categorized by Marler and Pickert (1984). See Fig. 2.3 for spectrograms. The first two rows represent the two tutor models at control (natural) performance levels (100%).

syllable type	# of notes/syllable	% of original	original trill rate (Hz)	high perf. trill rate (Hz)	freq. bandwidth (kHz)	note repetition rate (Hz)	final vocal deviation
626	3	100	7.6	7.6	3.763	22.7	2.83
2456	4	100	6.2	6.2	3.937	24.8	2.82
2512	4	115	6.5	7.5	3.296	30.1	3.30
6451	4	120	6.2	7.4	3.768	29.8	2.84
256	3	129	9.5	12.3	4.500	36.8	1.52
156	3	132	8.7	11.4	4.400	34.3	1.72
2351	4	137	9.5	13.0	3.965	52.1	1.96
2126	4	140	7.7	10.8	3.562	43.2	2.63
25	2	145	9.5	13.8	4.273	27.6	1.56
361	3	155	10.6	16.4	4.527	49.3	0.98



## Chapter 2 Figures

Figure 2. 1 Examples of vocal performance. a) A spectrogram of a wild-recorded swamp sparrow song. b) A “high-performance” version of this song, produced by trill manipulation. c) Vocal deviation measured from an upper bound (from Ballentine et al. 2004)

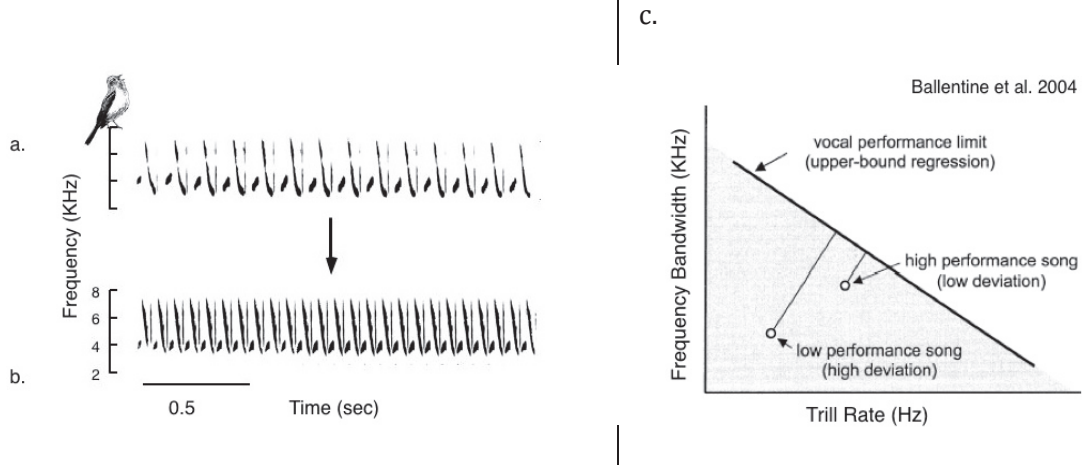


Figure 2. 2 Male mass gain over development. I measured individual male mass every four days to the nearest 0.1g. The nutritional stress manipulation began once sex was determined between day 7-10 (+/-1). Males fledged around 24 days, but I continued the hand feeding manipulation through day 40(+/-1). Results from the early nutritional manipulation through day 92 can be seen in Figure 2. Mass did not initially differ between groups, but by day 12, developmental stress males weighed significantly less than control males ( $t = -4.06$ ,  $p < 0.001$ ). Mass was also measured for all birds on 6 Nov. 2010, 19 Dec. 2010, and summer 2011.

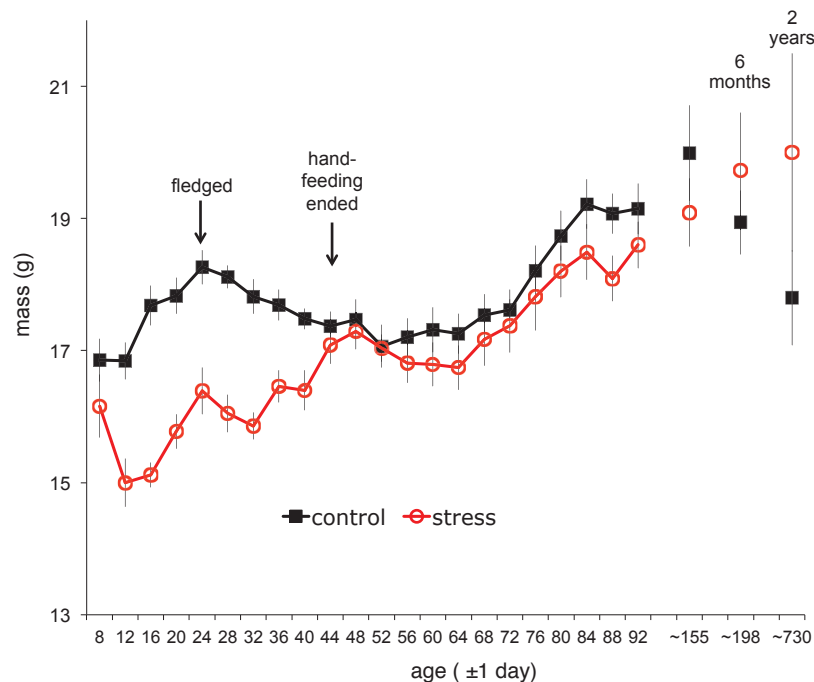


Figure 2. 3 Spectrograms of the ten tutor model songs including the percent trill rate relative to the original trill rate. Tutor models 5 and 8 were not increased in trill rate, and were maintained at the average of the wild-recorded trill rate. Songs used as tutor models naturally varied in note composition and the number of notes per syllable and original trill rate and frequency bandwidth (see Table 1).

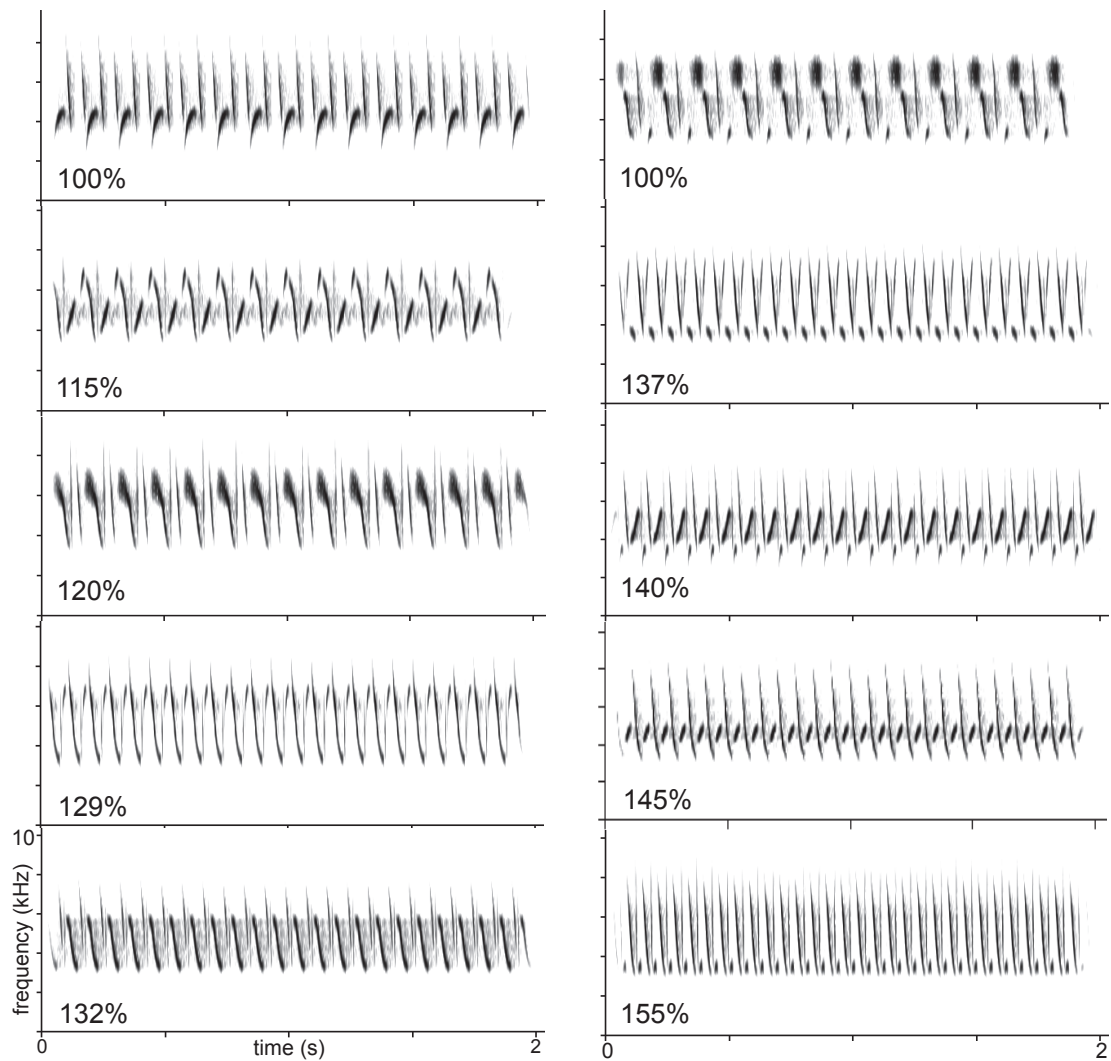


Figure 2. 4 (a-i) Example spectrograms of crystallized “copies” produced by the subject males in their first adult year (2010). These songs were determined by eye and spectral cross correlation of syllables and notes to be copied from the tutor models above. Rows above the copies within the same column depict the spectrograms of the tutor models from which they were copied. The same tutor model was often copied by different individuals. For example, the top left tutor model was copied by a control bird (a) and a developmental stress bird (b). The control bird copied all notes of the syllable but reproduced the song at a slower trill rate than the model, while the developmental stress bird reproduced the tutor song with a note omission, a decreased trill rate, and a shorter duration. Indeed most of the copies featured note omissions (b-g,i) and shortened durations.

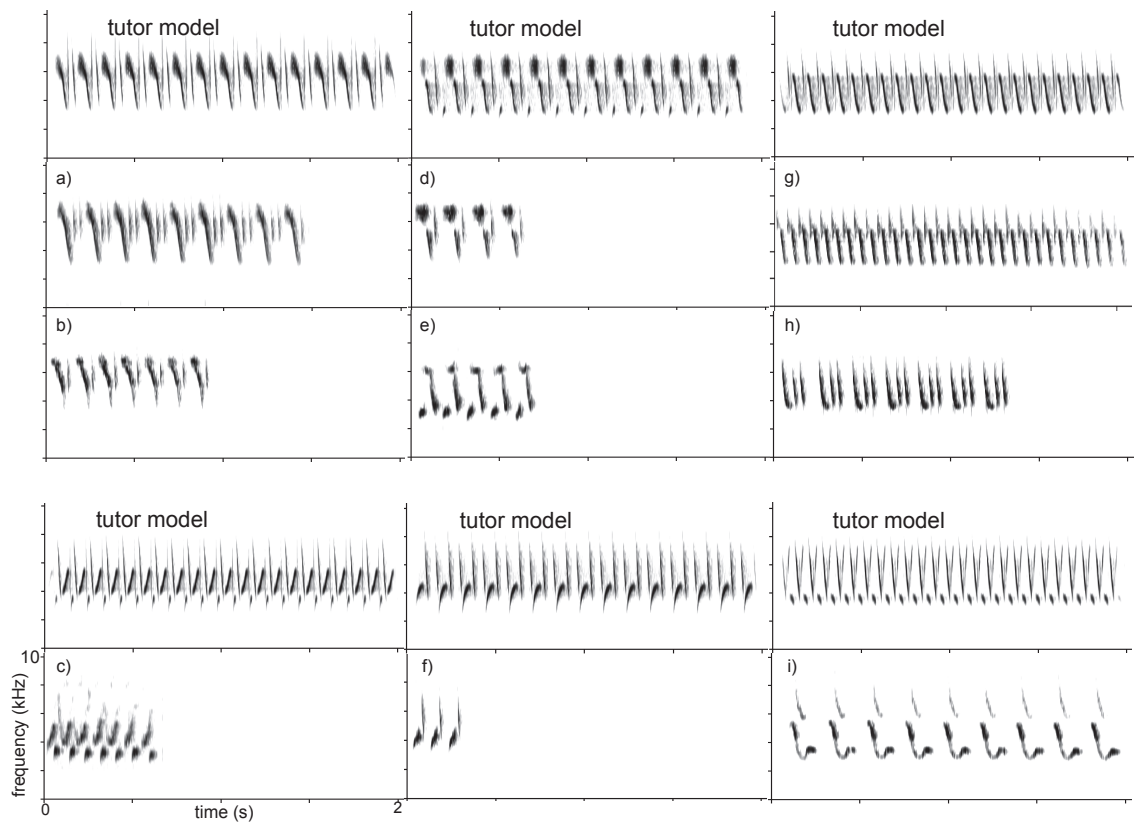


Figure 2. 5 Example spectrograms of “single-note trills” containing one note repeated in succession. Twelve of the fourteen males produced songs that only contained one note and were usually given at a rapid trill rate. Songs on the left column (control), songs on the right (developmental stress). SPCC matched trill notes to tutor notes resulting in matches averaging 76% (range 53-86%) maximum similarity to a tutor model note.

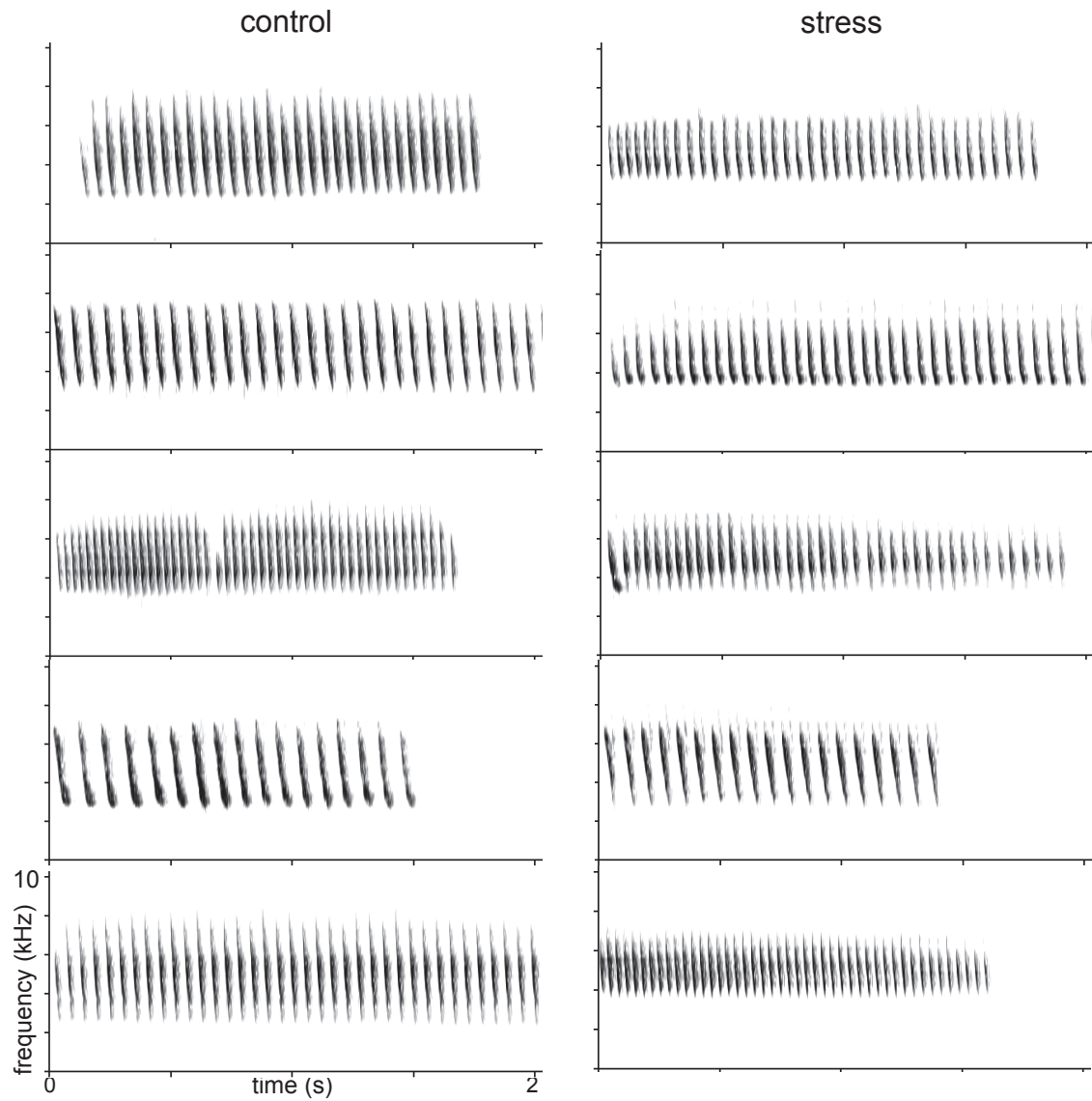


Figure 2. 6 Graphs of number of songs (copies and trills) produced by subject males (Y axis) against their matched tutor models (X axis) and grouped by tutor model modification percentage. a) Copies and trills appear to be similarly spread across the distribution of trill rate modification, with the tutor model increased the highest having been reproduced only as a one-note trill. b) control and developmental stress males matched tutor models across the distribution of trill rate modifications.

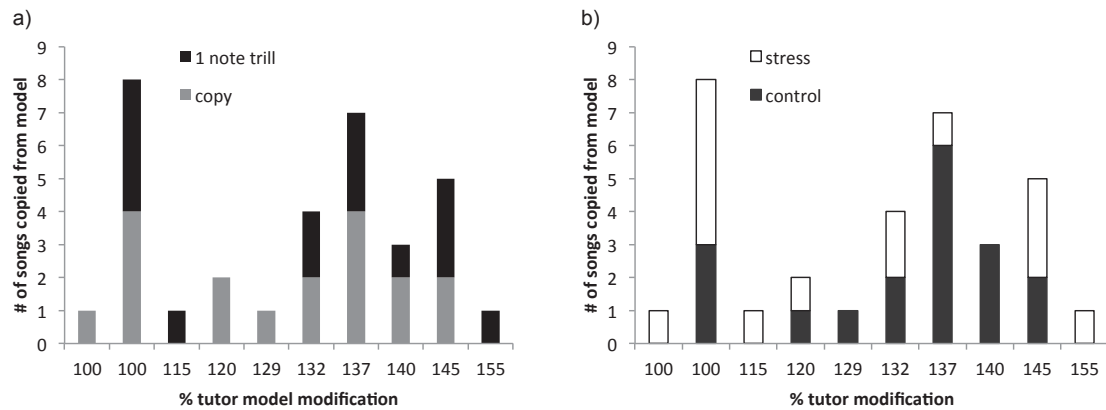


Figure 2. 7 Boxplots for copying accuracy as SPCC scores of matches to tutor syllables. The central line indicates the median, the edge of the box represents the 25<sup>th</sup> and 75<sup>th</sup> quartiles, and open circles indicate outliers. Copying accuracy of copied songs (syllables attributable to tutor model syllables, 1.00 indicating a perfect copy) was significantly higher for control songs (blue) than for developmental stress songs (red).

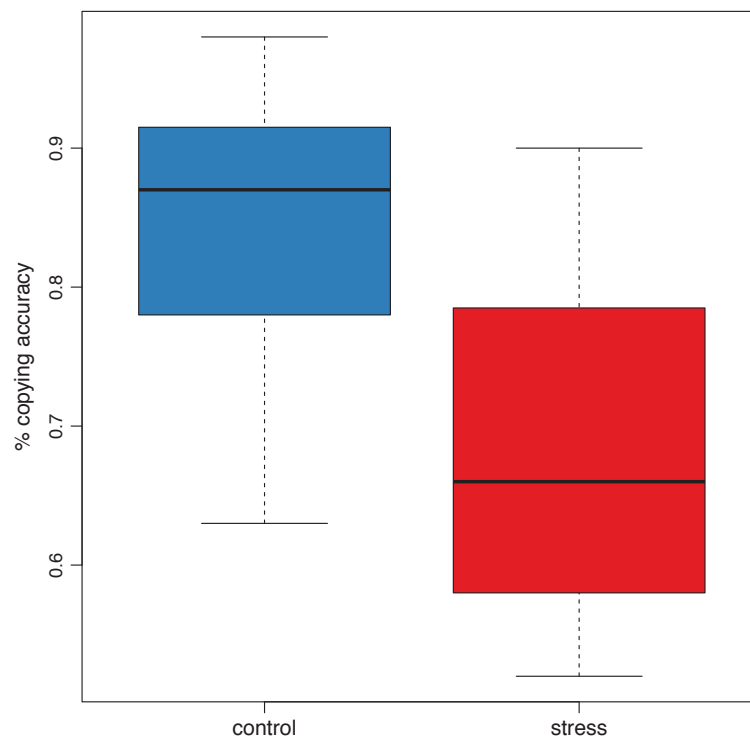


Figure 2.8 Boxplots for trill rate, frequency bandwidth, and vocal deviation for crystallized copies from the control and developmental stress groups for songs sung in 2010 (year 1). Trill rate showed no significant differences between groups for copies. Frequency bandwidth and vocal deviation differed significantly between groups ( $p < 0.05$ ).

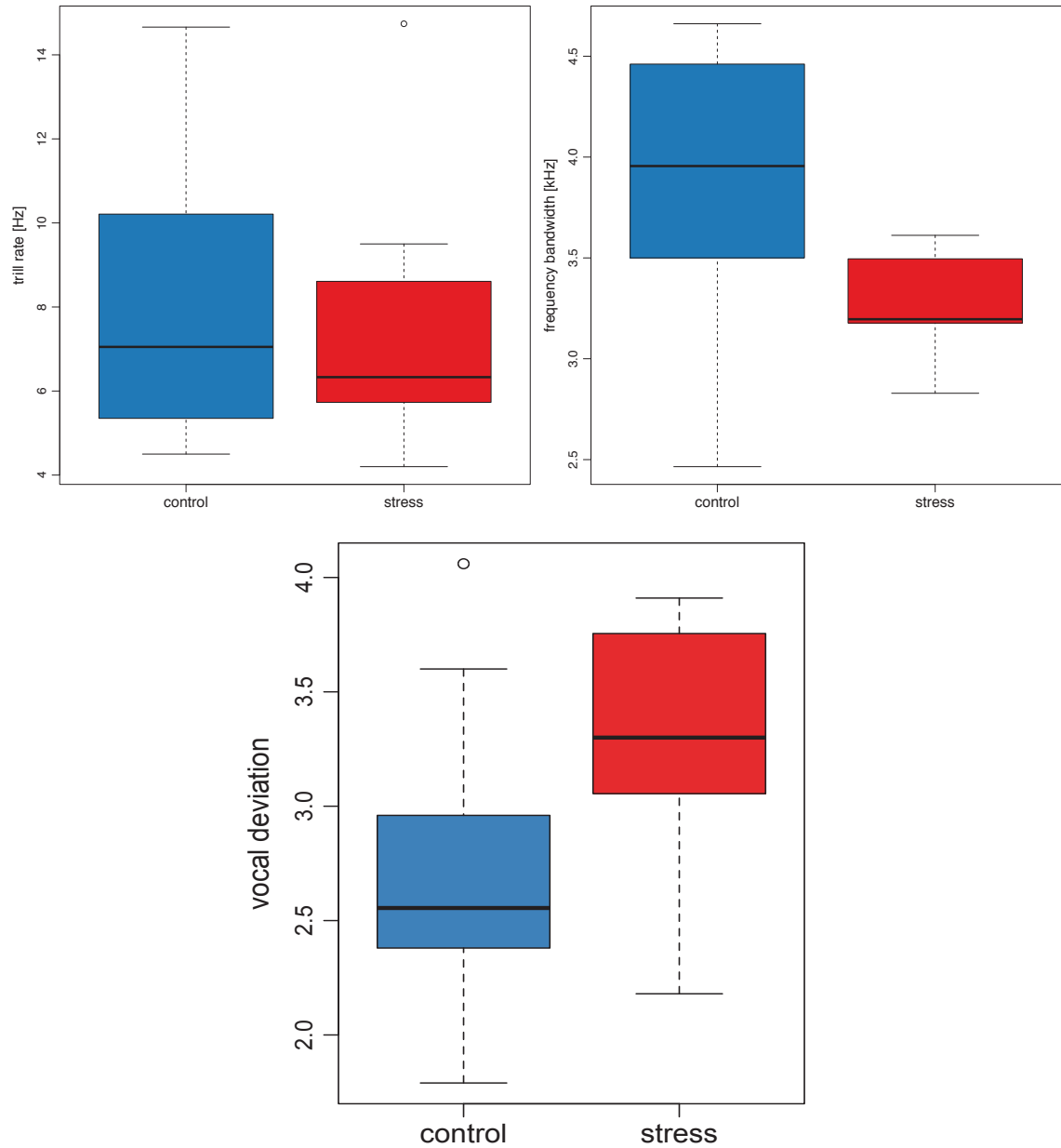


Figure 2. 9 Example spectrograms from individual subject males recorded in 2010 (top rows, year 1) and 2011 (bottom rows, year 2). Song types are matched across years and represented in the same column. Both copies and trills improved in vocal performance between years (see results and Fig. 2.10, 11).

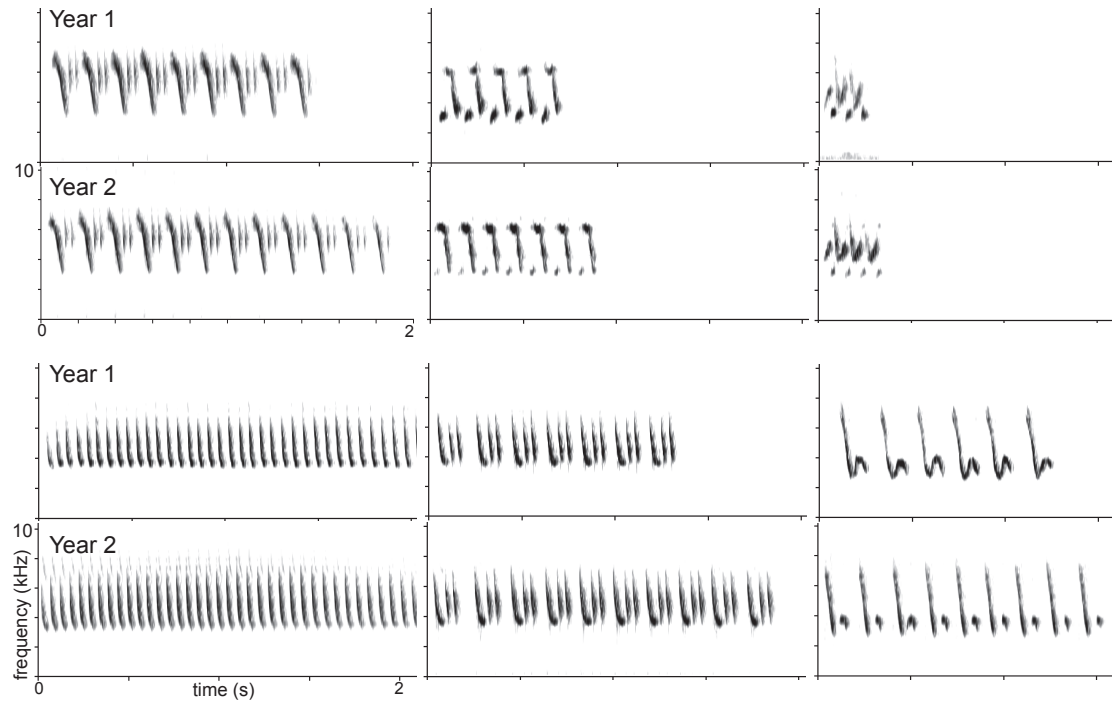


Figure 2. 10 Differences in vocal performance across years. A small vocal deviation indicates a higher vocal performance. Bars represent the difference in vocal deviation for individual songs from year to year (2011 minus 2010), zero would indicate no change, a negative value indicates vocal deviation decreased (performance increased), and a positive value indicates vocal deviation increased (performance decreased). Vocal deviation was significantly lower in the birds' second year.

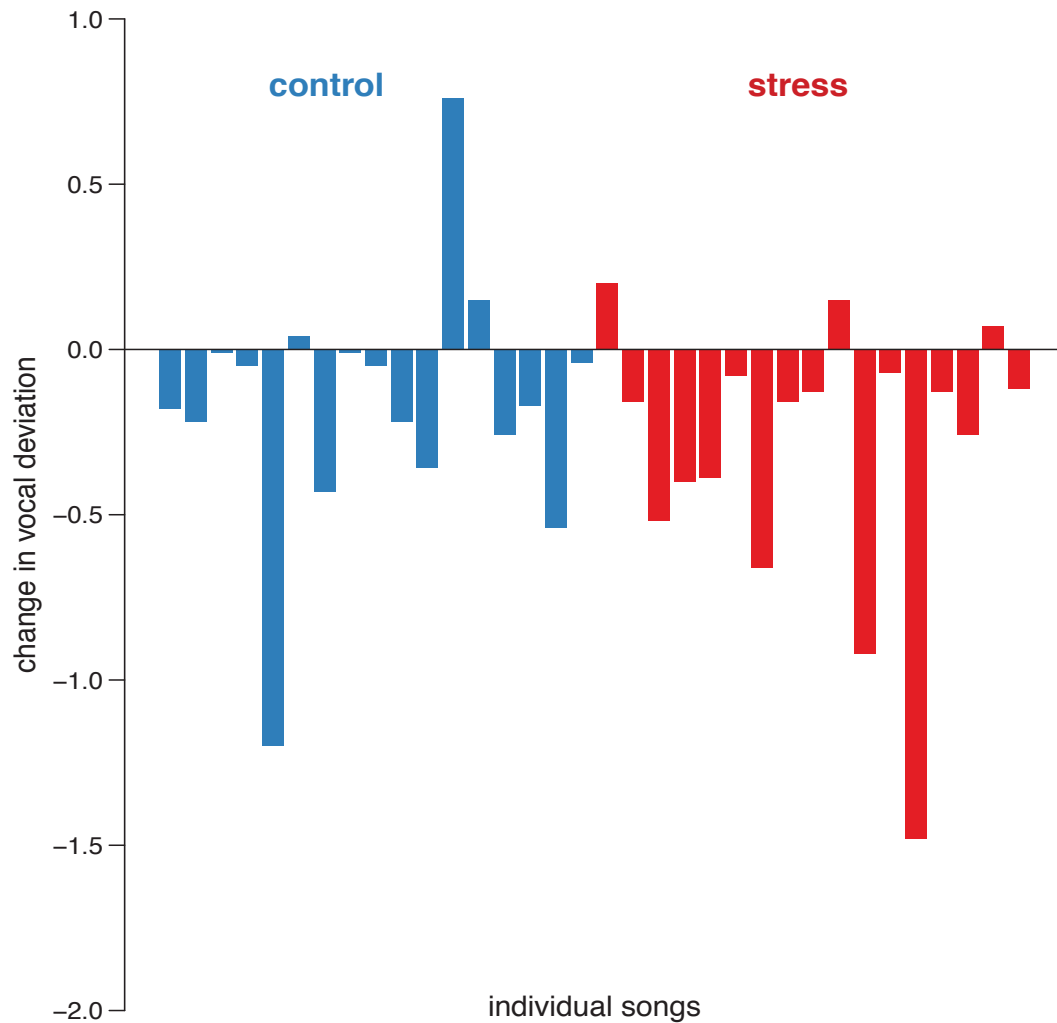
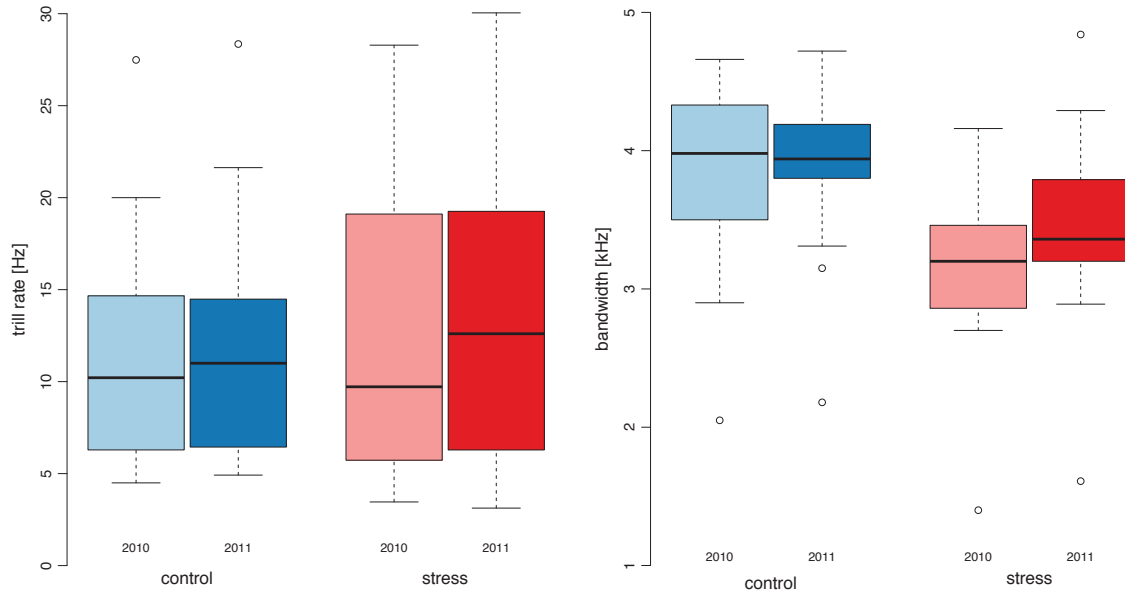




Figure 2. 11 Box plots of change in vocal deviation across years. Males of both groups improved their vocal performance by increasing both trill rate and frequency bandwidth, but the groups differed in which parameter increased at a statistically significant level. Control males significantly increased trill rate and developmental stress males significantly increased bandwidth. Results suggest that both groups seem to have improved their vocal performance through successive practice. The nutritional condition did not change for either group, but for developmental stress males more time had elapsed since their most severe nutritional stress.



**CHAPTER 3**  
**LEARNING AND INNATE BIASES GUIDE THE DEVELOPMENT OF**  
**FEMALE MATING PREFERENCES**

**Abstract**

Female mate preference is a crucial component of sexual selection, yet we have limited knowledge of how it develops. Mating preferences in adults may be shaped by multiple factors during development including genetic predispositions, social copying, and contextual learning. I studied female preference development in swamp sparrows (*Melospiza georgiana*), a species in which adult females are known to prefer songs characterized by high vocal performance. In two experiments, I raised and tutored females with song models of normal performance. Then employing a new method, I presented tutor songs paired with videos of adult females giving copulation solicitation displays (CSD) as further training. When birds reached breeding condition, I tested female preferences for familiar tutor songs against novel songs (Exp. 1) and then tutor songs against higher- and lower-performance versions (Exp. 2) using the CSD assay. To further draw out preferences of birds that did not display, I also retested a subset of the females in an operant conditioning assay in which females were able to elicit playback of songs by hopping on operant perches. In Exp. 1, females gave significantly more CSDs to tutor songs than to novel songs, and the direction of female preferences was stable in the operant assay. In Exp. 2 females gave significantly fewer CSDs to low-performance than to tutor songs at normal performance, and also responded most strongly to tutor songs overall, although at a level that did not differ significantly in pair-wise comparison to high-performance versions. In the operant assay, both high-performance and tutor stimuli were elicited significantly more than low-performance stimuli, but again high performance and tutor responses did not differ significantly. A greater response to tutor songs in both experiments implicates a

strong influence of experience in shaping female preference, although preference appears to also be driven by innate biases in favor of higher performance. These experiments provide the first evidence that female preference development for a sexually selected trait is guided by an interplay of learning and innate biases.

### **Introduction**

Understanding the origin and maintenance of female mating preferences has been called the foremost problem in the field of evolution by sexual selection (Searcy and Yasukawa 1996 p 454). To this day, the development of female preferences remains poorly understood. Traditionally mating preferences were considered to operate under the framework of a pure genetic basis. For example, runaway selection and sensory exploitation models of sexual selection imply that differences in female preferences are directed by differences in genotype (Fisher 1930, Kirkpatrick 1982, West-Eberhard 1984, Ryan & Rand 1990, Fuller et al. 2005). Yet several recent studies suggest that mate preferences can be shaped or modified by experience. Females of many species imprint on the traits of their parents, and retain preferences for those traits when they reach sexual maturity (reviewed by ten Cate and Vos 1999). While this research explains how females attend to cues in order to recognize their own species or specific individuals such as their mate, far less is known about how females discriminate among the traits of males within their own species. To what extent does female experience with male phenotypes influence mate choice?

The role of learning in female mate choice has historically received less attention, but recently studies have indicated its potential importance in sexual selection. There are a variety of types of learning including contextual, usage, comprehension, and production learning, and these types can occur in both signalers and receivers (Janik & Slater 1997, 2000). Females' contextual experience with male traits, in which females learn to associate

signals or traits with a particular context, can influence their mating decisions. To illustrate, female guppies (*Poecilia reticulata*) who experience dull male colors during early development, whether because of turbid waters or heavy predation on bright males, later prefer those duller colors over brighter, redder colors that are normally preferred in this species (Endler 1991, Breden & Stoner 1987). Other studies have shown females to use social information in the context of other females making mating decisions. Mature females appear to observe and then mimic the mate choices of other adult females, which were previously observed to choose males with certain phenotypes (reviewed by Freeberg 2000, Galef and White 2000). Recent studies by Hebets and colleagues more directly investigated the effect of females' direct experiences with male phenotypes early in life (Hebets 2003, Hebets and Vic 2007). Subadult female wolf spiders (*Schizocosa uetzi*) were exposed to males with one of two manipulated phenotypes (black or brown painted forelimbs) or not exposed to males at all (Hebets 2003). Prior exposure to either black- or brown-legged males resulted in a significant effect on mate choice with females choosing males with the familiar over non-familiar phenotype for copulation instead of cannibalism, while females not previously exposed to males showed no mate preference (Hebets 2003). These studies suggest females may retain a mating preference memory for phenotypes experienced early in life, and they lead to the question of whether or not females engage in comprehension learning. Comprehension learning is defined as occurring when a receiver comes to extract meaning from a signal as a result of experience with the usage of signals by other individuals (Janik and Slater 2000, pg. 2).

Within these realms, virtually nothing is known about how females develop preferences for males with complex signals or high-performance displays (e.g., Ballentine et al. 2004, Aguilar et al. 2008, Byers et al. 2010). Exploring the various innate and experiential contexts that define how behavior is learned may provide helpful insights into how females

develop preferences for high performance displays. Songbird studies, in particular, have provided unique opportunities to examine the dynamic interplay of learned and innate factors as they have evolved largely in the context of sexual selection (Catchpole & Slater 2008). A main finding is that song production development is guided by both experience and genetic predispositions. For instance, males tend to produce faithful copies of song models they hear through imitative learning, yet also show innate predispositions to copy conspecific versus heterospecific song models (Marler & Peters 1977, 1987). As for song preferences, it is well established that females of many songbird species assess prospective mates based on their songs (Searcy & Yasukawa 1996, Catchpole & Slater 2008), often preferring song variants of greater length (Wasserman & Cigliano 1991), complexity (Catchpole et al. 1984), or performance (Draganoui et al. 2002) to name a few examples. While sexual selection theory explains why females should attend to these signals, we still know little about how females develop preferences for certain song variants, and specifically how preference development is shaped by innate versus learned factors (e.g., Riebel et al. 2005).

Studies of wild birds provide only limited insight into these issues, mainly because of minimal information about individuals' prior social experiences and thus their effects on preference development (reviewed by Hernandez et al. 2008). In ground-breaking work with laboratory-reared birds, Riebel (2000, 2003) found that female zebra finches raised without tutor songs fail to develop normal preferences for specific male songs. In addition, zebra finches tutored early in life also show repeatable preferences for songs with specific levels of complexity (Lauay et al. 2004). These findings suggest a role for acoustic experience in shaping preference development, perhaps during a sensitive period of development parallel to that already shown for song memorization in young males (Marler

& Peters 1987). Yet it is plausible that, as with song learning in males, innate factors also bias the process by which experience shapes female preference development.

One aspect of complex mating displays like song that seems favored by sexual selection is display performance, because only high-quality males should be able to execute complex displays with precision and consistency (Byers et al. 2010). In the study of bird songs, females of several species demonstrate preferences for trilled songs with high vocal performance. More specifically, canaries (*Serinus canaria*), Lincoln's sparrows (*Melospiza lincolnii*), and the focal species here, swamp sparrows (*M. georgiana*), all have been shown to respond preferentially to trilled songs with faster trill rates or wider frequency bandwidth (Vallet & Kreutzer 1995, Draganoiu et al. 2002, Ballentine et al. 2004, Caro et al. 2010, see also Podos 1997, Podos et al. 2009). We do not yet know, however, the extent to which these preferences were shaped by past social and acoustic experiences, or by potential innate biases for high-performance songs.

Experimental tests of song preferences require a biologically valid assay of song preference. A favored method is the “copulation solicitation display” (CSD), in which a female bird stimulated appropriately arches her back, raises her head, and exposes her cloaca (Baker et al. 1981, see Fig. 3.1). CSDs are given as a prelude to copulation in nature, and are thus regarded as a clear indication of mate preference (King and West 1977, Searcy 1992). In many species, wild-caught birds implanted with estradiol present CSDs in response to playback of some songs and not others, thus allowing insight into their preferences (e.g., Ballentine et al. 2004). Yet, prior studies on lab-reared females have almost never managed to elicit CSDs from hand-reared females (cf. Baker et al. 1981), and have instead relied on metrics of preference such as association tendencies and phonotaxis. It seems that females require some amount of socialization or contextual cues, or that the lab environment deprives them of some other necessary aspect, in order to respond with

CSDs to preferred songs. This difficulty has obstructed experimental tests of how experience may shape preference development, because only in the laboratory can birds' social experiences be controlled and documented.

To solve this problem I designed and validated a new method that primed hand-reared females to perform CSDs. In brief, I raised female nestlings and presented them with a regime of model songs during their first summer. During the early spring, I then presented the same regime of tutor songs played in tandem with life-sized videos of adult females presenting CSDs. I posited that coupled audio and video images would model CSD behavior and context for young females, and thus increase the likelihood that they would themselves perform CSDs upon maturity. When tested in summer, almost all of these females indeed performed full, normal CSDs (see results below, Figs. 3.1, 3.4). Another recent method employs operant conditioning in which perches are linked to song stimuli and birds can elicit these stimuli as they choose (Riebel and Slater 1998, Riebel 2000, Holveck and Riebel 2007, Anderson 2009). These new methods provided a novel opportunity to examine factors involved in female preference development.

What is the relative influence of learned versus innate factors in preference development? I address this question in two experiments on song preference development in female swamp sparrows, *Melospiza georgiana*. In the first I presented females with familiar (tutor) versus unfamiliar (non-tutor, novel) exemplars of swamp sparrow song. If preferences are guided by learning, we would expect birds to respond more strongly to playback of familiar songs. In the second experiment I presented females with their tutor songs, which were at normal-performance levels, against low- and high-performance versions of these songs. I hypothesized that females would prefer high-performance versus low-performance songs, parallel to results from Ballentine et al. (2004). Such a preference, if demonstrated, would be interpreted as an innate bias given that birds had no experience

with either low- or high- performance song stimuli. This second experiment also allowed me to assess the relative influence of innate bias for performance versus the influence of experience, given that the normal-performance stimuli were familiar tutor songs.

## **Methods**

### **Animal care and song training**

In the summers 2007 and 2009, juvenile females were taken from the Quabbin Reservoir, Franklin County, MA, between the age four and eight days old, and reared in the lab (as in Podos 1996 & Lahti et al. 2011). Nestlings were collected throughout the summer with females hatching from the end of May to the end of July. The age of all females was determined to within one day of the hatch date. Fourteen females were raised to adulthood from the 2007 cohort, and twelve from the 2009 cohort. Females were trained in two phases. In the first phase, females were presented with a training regime of ten normal tutor songs (Figs. 3.2ab, 3.3d-f) for an hour, twice daily at 0700H and 1800h. Phase 1 occurred from when females were aged  $10 \pm 10$  days through  $120 \pm 14$  days old, which corresponds in timing to the sensitive period for song acquisition in male swamp sparrows (Marler & Peters 1977, 1981). Tutor songs were prepared from wild-recorded songs of known males in the local population. I chose ten distinct song types that were representative of the vocal diversity in the population (Marler & Pickert 1984). In order to control for within song consistency, I measured the average trill rate, which was the average syllable length for eight syllables, then selected a syllable in the middle of the song and concatenated this syllable into a two second song at the average trill rate (see song stimuli preparation below).

The second phase occurred the following spring in March, when females were aged between 260-310 days old. Females were presented with the same ten model songs as



previously, but this time coupled to videos of adult females either performing CSDs or sitting on a perch. Videos were presented on high definition LCD monitors (Samsung LN-T1954H) and tutor songs were played from speakers (Advent Power Partner AV570 70-Watt) near the video monitors (Fig. 3.1b). Displays were synchronized to follow directly after each song, mimicking the timing of natural responses. Half of the females ( $n = 5$ , 2008,  $n = 6$  2010) were presented with a video of CSDs coupled with tutor songs #'s 1-5, while the other half of the females ( $n = 5$ , 2008;  $n = 6$  2010) were presented with the CSDs video coupled with tutor songs #'s 6-10. Tutor songs not coupled with CSD videos were instead paired with videos of perched adult females. Video stimuli were presented at least 3 times a week for 3 weeks.

In order to test and control for the effect of this method on song preferences, I tested for a preference for tutor songs paired with the video of the displays versus tutor songs paired with a video of a perched female. This test also addresses the potential for mate-choice copying as females experienced a simulation of adult females showing preferences for certain male song types. Subjects were ten females from the 2007 cohort.

Additionally in 2005, eight females were raised to adulthood with similar methods but without video training. All rearing and research protocols were approved by the UMass IACUC (Protocol #24-10-12).

### **Copulation Solicitation Display Assay**

In June and July of the nestlings' second summer (ages 330-360 days), I conducted preference trials following methods of Ballentine et al. (2004). At least two weeks before testing, females were brought up to 15:9 h light:dark cycle to stimulate breeding condition. To ensure sexual receptivity, ten days before testing I implanted the birds with 17 $\beta$ -estradiol in silastic tubing of 1.96-mm outer diameter and 1cm long (Searcy 1992). Playback trials took place in individual acoustic-isolation chambers (Acoustic Systems) that

contained a loudspeaker connected to an iPod in an adjacent room. The entire cage containing the female was placed in the individual chamber three days before testing to allow for acclimation. For each trial, females were presented with three minutes total of either two different song types (Experiment 1) or three different song types (Experiment 2), with songs presented at a rate of 6 songs per minute. Trials were filmed with video cameras positioned outside chamber windows. All song types were normalized for amplitude in Signal and presented during testing at an amplitude of  $60 \pm 2$  dB, as measured by a sound pressure meter.

### **Experiment 1: tutor versus novel stimuli**

Trials contrasted playback of familiar tutor songs versus novel songs (Fig. 3.2). Tutor and novel stimuli were matched for vocal performance levels in trill rate and notes/syllable, to minimize any effect of possible preferences for songs of high performance (see Exp. 2). I used paired t tests to confirm that tutor and novel songs did not differ in trill rate, frequency bandwidth, and vocal deviation (all tests  $p > 0.4$ ). Females had not previously experienced the novel songs, but all novel songs were recorded from the same local population as the tutor songs. Each female received a unique set of two tutor song types matched to two novel song types. Females were tested in four trials over two days, with only tutor songs or only novel songs in the morning trial, and the opposite category 3hrs later. The order of presentation was reversed on birds' second day, and overall presentation order was counter-balanced across females. Individual female responses to each stimulus category were summed over the 2 days of testing to account for any presentation order effects (Nowicki et al. 2001, Ballentine et al. 2004). Subjects were included in the analysis if the female gave at least one CSD in any of the four trials ( $n = 13$  females combined across the two rearing cohorts).

## **Experiment 2: performance versus experience**

The subjects were eleven females all from the 2009 cohort. Trials contrasted sets of normal- versus low- versus high-performance stimuli. Normal performance playback stimuli were constructed from the original tutor songs, which naturally ranged in trill rate between 4.25 - 9.52 Hz and in vocal deviation 1.89 - 3.20. I then constructed low- and high-performance versions of tutor songs, by either decreasing or increasing trill rate (Fig. 3.3). Manipulated stimuli ranged from 3.4 - 7.14 Hz in trill rates and 2.18 - 3.35 in vocal deviations for low performance and 4.89 - 11.4 Hz and vocal deviations 1.66 - 3.12 for high performance. Each female received a total of six trials over three days, two trials per day with 3hrs between trials, with each category of stimulus presented twice, in a counter-balanced design across females.

### **Operant Assay**

To further draw out female preferences for those birds that did not display and to test for any subtle differences between preferences for high and normal performance, I re-tested nine females from the 2009 cohort with an operant conditioning assay in June and July of 2012. I generally followed the methods developed for swamp sparrows by Anderson (2009), which are similar to those used by Riebel and colleagues (Riebel and Slater 1998, Riebel 2000, Holveck and Riebel 2007). Cages and individual acoustic chambers were the same as those used in the CSD assay, but contained two speakers, one on either end of the cage, next to two operant perches linked to infrared sensors (Fig. 3.1d). The two operant perches were on opposite ends of the cage roughly 38 cm apart, and were each connected to a sensor that detected when birds alighted along a perch within roughly 14cm of the sensor. The sensors were connected to a PC computer running software which logged the time of each arrival on the perch, noted the identity of the perch (left or right), and triggered playback of one song from the speaker on the corresponding side of the cage (McClure

2013). A third perch in the center of the cage was not connected to a sensor, and similarly all other locations along the floor and sides of the cage would not trigger the sensors. Females remained in the operant cage at all times during training and also during testing, but were returned to regular cages between training and testing. In order to cue the females that the program was on and it was possible to elicit playback, small LED lights, one within 2 cm of each sensor, were programmed to turn on when the perch-sensors were active.

In May, the nine females were trained for 6-10 days on the operant perches in order to learn that the two perches elicited playback of different song types. Training sessions occurred for 3 hrs in the morning and evening between the hours of 0600-1200 and 1430-2030 with three hours of inactivity between trials. Females were trained with two sets of two song types from their original tutor set that had not been used in CSD testing, one song triggered by the left and the other song triggered by the right perch. Females were given a “forced task” in training such that they had to sample each side four times before both perches were active for free choice. During this forced task, one perch was active at a time, which was indicated by its LED light. The software was programmed to activate a perch at random for four times, and then activate the opposite order of perches for the next four hops. A perch had to be visited before the next perch would become available. After the eight total forced perch hops, both perches became active and females were able to freely hop on either perch for a sum of 70 times before another forced trial would begin, and this occurred in loop. Every three sessions I switched the sides of the stimuli in order to disassociate a song with a side, and generally to avoid side biases. After four days of training, I switched the stimuli to the other set of two tutor songs. Females were trained without estradiol implants, but were implanted after training had been completed. I tested females 14 ( $\pm 4$ ) days after implantation. All stimuli used in the operant training and testing

were the original playbacks of tutor and novel song types and of low, normal, and high performance song types, all  $2 \pm 0.1$  sec in length at 60 ( $\pm 2$ ) dB.

### **Operant testing Experiment 1:**

Females were tested with two contrasting sets of tutor versus novel stimuli, with one song type of each category elicited from the left perch and one from the right perch per trial. The stimuli used were the same four stimuli that the female had received in the CSD assay consisting of two tutor songs and their two matched novel songs. Note that the females had previously experienced the novel song in CSD testing. Each female received four trials across two consecutive days of testing with trials in the morning and afternoon during the same hours as training was conducted. Stimuli sets were counter balanced across sides such that the females received a tutor stimulus from the left and a novel stimulus from the right once and vice versa once for each of the two stimuli sets. The same software was used which programed 8 forced: 70 free hops, to ensure that each side was sampled. If a female made fewer than a total of 60 hops to perches in a trial, then that trial was repeated immediate after the four testing sessions. Forced trial hops were removed from the data before analysis.

### **Operant testing Experiment 2:**

Females were tested in the same manner as in Exp. 1 except as follows. Females were tested with sets of contrasting stimuli of high versus normal versus low performance for 3 consecutive days, two trials per day in the morning and evening. During each operant trial, two stimuli (one on each side) from the three performance categories were available to be triggered by the operant perches. I selected one song type from in each female's previous CSD performance experiment and its low and high performance versions to use in the operant test ( $n = 27$  stimuli). I presented the same order and combination of contrasting stimulus sets that females had received in the CSD experiment. For example if a female had

received high performance in the morning and normal performance in the afternoon on day 1 of the CSD assay, then her first operant testing trial was the high performance stimulus from the left and normal on the right perch (Table 1). For each new trial I kept one stimulus on the same side and switched the other to the next performance category beginning with switching stimulus on the left (in the above example to low, for a low vs normal trial). I tested each combination twice, but switched the sides from which the stimuli pairs were presented originally for the second round. As in the CSD assay, the order of presentation was conducted using a counter-balanced design across females, and additionally the sides of the presentation were counter balanced.

### **Statistical analyses**

For the copulation solicitation assay, videos of birds' responses to song playback were scored by two observers blind to stimulus treatment. For each trial, observers identified each CSD given and assigned each a score (1-5) to reflect display degree and duration. CSD scores were summed within trial, averaged across trials within each treatment, and then square-root transformed. In Exp. 1, I tested for response differences between tutor and novel stimuli using a Wilcoxon matched-pairs signed-ranks test. In Exp. 2, I used a Friedman test to identify overall differences in responses to performance stimuli treatments, and then, in lieu of a Tukey's HSD test, I assessed differences among treatment pairs using the nonparametric Wilcoxon matched-pairs signed-ranks tests. For the operant assay, I used the same statistical analyses as in the CSD assay but this time testing the number of perch hops. All analyses were conducted in *R*.

## **Results**

### **New Method and CSD Assay**

In the two cohorts for which I employed the video CSD training method, eight of ten females (2007) and eleven out twelve females (2009) gave CSDs in response to song playback. These displays were similar in number and magnitude as seen in laboratory tests of wild-caught adult females with individual females giving an average of 3.7 (range 0 – 18) CSDs per treatment (Ballentine et al. 2004, Anderson 2009). By contrast, in tests with hand-reared females without the video-training method (2005), none of the eight females gave CSDs in response to male song stimuli. Other researchers have similarly observed that females fail to give CSDs when raised in the lab without exposure to videos or live males (Nowicki, Peters, Ballentine, pers. comm.). Females showed no difference in preferences between tutor songs paired with or without videos of displays (Wilcoxon matched pairs signed-ranks test  $n = 8$ ,  $\beta_{(\text{display} - \text{no display})} = 1.1$ ,  $P = 0.49$ ), suggesting that the video training did not influence song preferences.

### **CSD Experiment 1: Experience – Tutor versus Novel**

Females gave more numerous and robust CSDs in response to tutor than to novel stimuli (Fig. 3.4a, Wilcoxon signed-ranks test:  $Z = 2.01$ ,  $N = 13$ ,  $P = 0.04$ , Effect Size:  $\beta_{(\text{tutor} - \text{novel})} = 1.6$ ). Females performed CSDs in response to both stimulus categories, but 11 of 13 females responded more strongly to tutor than to novel song.

### **CSD Experiment 2: Performance – Tutor songs versus low- and high-performance versions**

Female responses varied significantly by treatment (Fig. 3.4, Friedman test:  $Z = 6.5263$ ,  $df = 2$ ,  $N = 11$ ,  $P = 0.038$ ; Effect sizes:  $\beta_{(\text{normal-low})} = 6.5$ ,  $\beta_{(\text{high-low})} = 3.5$ ,  $\beta_{(\text{high-normal})} = -3$ ). Females gave significantly fewer and weaker CSDs to low-performance versions than to normal-performance tutored songs (Wilcoxon signed-ranks test:  $Z = 2.17$ ,  $N = 11$ ,  $P = 0.023$ ).

Females also responded with the most CSDs to tutored songs overall, but this value did not differ significantly from high-performance songs (Wilcoxon signed-ranks test:  $P > 0.1$ ).

Females varied individually as to which stimulus category they preferred. Five of eleven females displayed most robustly to normal performance (tutor songs) while four females displayed the most to high performance. Two females gave equal responses to tutor and to high-performance songs. No female responded the most to low performance.

### **Operant Assay**

All females learned to elicit song from the operant perches. Females varied in the number of visits they made to operant perches. Five out of nine females also performed CSDs in response to songs elicited upon hopping on a perch.

#### **Operant Experiment 1: Experience – Tutor versus Novel**

The nine females varied in the number of hops they made to the operant perches (mean=263.8, range = 63 - 1803). Females made more visits to perches eliciting tutor song, but this did not differ significantly from visits to novel song perches (Fig. 3.4b,  $\beta_{(\text{tutor-novel})} = 72.4$ ,  $W = 29$ ,  $P = 0.074$ ). Females had previous experience with these novel songs in their CSD trials. Overall the direction of female preferences was stable in that six of the nine females hopped more on tutor than on novel songs perches, one female was tied, and two females hopped more on novel songs perches.

#### **Operant Experiment 2: Performance – Tutor songs versus low- and high-performance versions**

Females varied in the number of hops they made per trial, ranging from 94 – 1673 hops (mean = 640.1). For the nine females tested in Exp. 2, the number of perch hops varied by performance level, with females preferring the two higher performance stimuli (Fig. 4,  $V = 12.67$ ,  $N = 9$ ,  $df = 2$ ,  $p = 0.0018$ ). The lowest number of hops were made to low-performance stimuli and this amount was significantly different from hops that triggered



either the normal- or high-performance versions ( $\beta_{\text{(normal-low)}} = 244.3$ ,  $V = 40$ ,  $P = 0.039$ ;  $\beta_{\text{(high-low)}} = 359.7$ ,  $V = 45$ ,  $P = 0.004$ ). High-performance perches were visited the most, but this was not significantly different from normal-performance perch visits ( $\beta_{\text{(high-normal)}} = 115.3$ ,  $V = 36$ ,  $P = 0.129$ ).

## **Discussion**

In two experiments, I tested the following hypotheses: First, experiences during ontogeny, particularly those involving learning, may guide the development of female preferences; and second, innate biases for sexually selected traits may guide the development of female preferences, and may be robust to specific experiences.

In Exp. 1, females gave significantly more numerous and robust CSDs to tutor songs than to novel songs, indicating a preference for song types experienced early in life in the CSD assay (Fig. 3.4a). Tutor and novel songs were both recorded from the same local population, and were matched for similar note, syllable, and vocal performance features, and therefore only differed in whether they were presented to females in their rearing regime. Females therefore appear to learn song types early in life, and those memories guide later song preferences. Previous research by Nowicki and colleagues (2002) found that adult females prefer songs of males that produced better copies of model songs to those of males who copied poorly. Taken together, these results imply that females retain memories for species-specific song that persist into adulthood, and are able to assess if songs match a tutor model. Song memories are perhaps preserved in neural templates, similar to those that guide song learning in males (Marler 1976, Prather et al. 2010). These results also suggest that like males, females have “preference template”, constructed during early development, which then would be used to distinguish certain songs from others not previously experienced. While females of many species of temperate birds do not learn

songs to produce, females may memorize song models to prefer as adults. An experiment on northern cardinals (*Cardinalis cardinalis*), a species in which females do learn to produce complex songs, however, showed the songs females selected for song production were not preferred in the sexual context, although whether or not tutor songs were generally preferred over novel songs was not tested (Yamaguchi 1999). Results in the present study are suggestive of an ability for comprehension learning and further song-model learning in females. Song memories for preferred tutor songs against novel songs could be further tested in neurophysiology experiments measuring responses in song memory regions of the brain.

Females swamp sparrows did respond to novel songs to some degree, which suggests that the template can be generalized beyond songs learned early in life. When females were tested with the operant assay, they were presented with the same tutor song versus novel song stimuli, and, although it had been one year since females had been exposed to the novel songs, they had previously experienced these novel songs in CSD testing. The majority of females (six out of nine) visited perches that elicited tutor songs more than novel songs although at a level that was not statistically significant (Fig. 3.4c). Preference differences between tutor and novel songs may have decreased after some familiarity with novel songs. Male song appears to be inherently rewarding to females (Anderson 2009), and thus females may choose to elicit song if it appears to be a viable signal. In this way learning may function under sexual selection such that males singing songs that are either copied poorly or do not follow the general song structure that females experienced early in life would not be chosen as mates. Indeed, in a study testing female preferences for normal conspecific song versus conspecific songs with innovations, females were seen to be conservative in their preferences and displayed significantly more to normal than to innovative songs (Nowicki et al. 2001). Potential follow-up questions would

address if learning models apply to females' assessment of copying accuracy, geographic similarity (Anderson 2009), or conspecific versus heterospecific signals. Much remains to be learned as to whether the role of learning in mate choice leads to stabilizing selection in the cultural and genetic evolution of male signals.

In Exp. 2, females received playback of contrasting sets of low versus normal versus high performance versions of the original tutor songs. Tutor songs were originally presented during rearing at the normal-performance level, which was the wild-recorded trill rate. In the CSD assay, females gave the weakest CSD responses to low-performance stimuli (Fig. 3.4b). Normal-performance levels elicited the highest CSD responses, which were significantly higher than responses to low performance, but display strength that was given in response to high-performance songs did not differ significantly from that given to normal songs. Results were similar in the operant assay, with females eliciting both high- and normal-performance stimuli significantly more than the low performance stimuli (Fig. 3.4d). Again responses to high and normal performance did not differ, but this time the most visits were made to the high-performance perches. These results suggest an innate bias for normal- to high- vocal performance. Further the asymmetrical response to low versus high performance versions suggests that female preferences may drive male signal evolution via an upward selective pressure on male vocal performance.

Previously, females captured as adults have been shown to prefer higher performance songs of those found in nature (Ballentine et al. 2004), but the question remained as to whether this preference was guided by past experiences. Past research on swamp sparrows suggests that trill rate is also a salient feature in song function for territorial males (Liu et al. 2008, Ballentine 2009, DuBois et al. 2009, 2011, Moseley et al. 2013). Females are thought to prefer high-performance songs because only high-quality males are likely to achieve high performance (Byers et al. 2010). Indeed vocal performance

has been suggested as an intersexually selected trait in a number of bird species (Vallet & Kreutzer 1995, Draganoiu et al. 2002, Ballentine et al. 2004, Caro et al. 2010). Given the results from Exp. 2, preference for higher performance may be innate, but also mediated by song learning as tutor songs were originally presented at normal-performance levels and were responded to as strongly as high performance. My results show evidence of weak or no preference for low performance, but that preference for high performance is balanced by a preference for the trill rate experienced early in life. These results parallel those found in a recent study of male song learning in swamp sparrows by Lahti and colleagues (2011). In this study, males tutored with songs of low, normal, and high performance also rejected the very lowest-performance song models, but copied other models with a balance of copying accurately and increasing performance level (Lahti et al. 2011).

In the present study, evidence that females prefer both songs experienced early in life and songs of relatively higher performance suggests an interplay of learning and innate biases. Indeed the proposed hypotheses are not mutually exclusive, and both fundamental factors are likely to work together in shaping female preference development as it relates to male signaling performance. The extent to which learning may facilitate a directional bias in preference or whether this directionality is governed by purely genetic biases is crucial to an understanding of sexual preferences and subsequent trait evolution (Verzijden et al. 2012). Turning to how female preferences affect signal evolution in swamp sparrows, results from these experiments imply that females would reject males with very low performance and prefer males singing songs that are both higher performance levels and better match song models experienced early in life.

Broadly, experiments in other taxa have shown positive effects of females' recent experiences with males, but the majority of this literature focuses on females' adult experiences; examples show support for mate-choice copying (reviewed by Galef and

Laland 2005), and preference for familiar males in sticklebacks (Bakker and Milinski 1991) and birds (Collins 1995). Effects of experiences during ontogeny have mostly tested the bounds of species recognition, and until recently most of the literature has focused on sexual imprinting (reviewed by ten Cate and Vos 1999). Few studies have shown support for the modification through development of female preferences for intraspecific male phenotypes, specifically modification with respect to learning (reviewed by Verzijden et al. 2012). For example early exposure to male phenotypes has been shown to affect the preferences of spiders (Hebets 2003), such that females tend to prefer the phenotype presented early in life. Moreover results in the present study suggest that female swamp sparrows can discriminate between the subtle differences of song variants all from a local population with similar performance and note structure. Even less well understood is how females come to prefer complex signals, and how preferences for high signal values such as high-performance displays come to develop. Theoretical models have recently been developed to explain how female preferences for complex signals may arise through genetic and cultural evolution (e.g. Kokko et al. 2002, Laland 1994). An empirical example in recent study on two syntopic species of wolf spiders shows that females appear to prefer ornamented males whom perform courtship displays over non-ornamented male forms regardless of the phenotype with which they had prior experience (Hebets and Vic 2007). Open questions of the origin, maintenance and development of female preferences for male signals remain.

The new video-training method holds exciting potential for investigating how mate preference develops in female songbirds. In two years of employing the video-training method, normal CSD behavior was elicited in 19 of 22 females. Results from testing preferences for songs trained with display videos or without display videos suggest females of this species do not engage in mate-choice copying, at least not with the level of social

information provided in the training videos. These results also suggest the method can be used without overly influencing preference for songs presented during video-training. This video method could be applied to questions regarding the plasticity of female preferences and address the extent of species-recognition for song or preferences for innovation or signal stability. Additionally the operant assay enables tests in which females can simultaneously compare stimuli and elicit these songs at will. Operant and CSD assays were shown to be congruent, giving similar results of direction of preferences both within and among individuals (Anderson 2009). Combining the video-training method with either CSD or operant assay would likely provide reliable assessment of female preference development.

### **Conclusions**

Generally, little is known about the factors that guide the development of female preferences. The extent to which female preferences are governed by genetic biases and by experiential and learned factors will influence signal evolution in male traits. Ultimately understanding the evolution and development of both signal senders and receivers will unite what have often been separate research aims in the fields of sexual selection and animal communication, and will yield valuable generalizations about trait evolution in signaling systems.

Table 3. 1 Example of stimulus presentation. Stimuli were paired in contrasting sets of two, with one stimulus elicited from the left and one from the right. Every combination of two from the three stimuli categories high, normal, and low performance was tested and from each side of the cage.

<b>Bird</b>	<b>Trial</b>	<b>Side</b>	
<b>female 1</b>		<b>Left</b>	<b>Right</b>
<b>day 1</b>	AM	high	normal
	PM	low	normal
<b>day 2</b>	AM	low	high
	PM	normal	high
<b>day 3</b>	AM	normal	low
	PM	high	low
<b>female 2</b>			
<b>day 1</b>	AM	low	high
	PM	normal	high
<b>day 2</b>	AM	normal	low
	PM	high	low
<b>day 3</b>	AM	high	normal
	PM	low	normal
<b>female 3</b>			
<b>day 1</b>	AM	normal	low
	PM	high	low
<b>day 2</b>	AM	high	normal
	PM	low	normal
<b>day 3</b>	AM	low	high
	PM	normal	high

### **Chapter 3 Figures**

Figure 3. 1 Copulation solicitation display (CSD) behavior. a) an illustration of a CSD (by D. Moseley adapted from Baker et al. 1981); b) a still image from the training video presented to young females in early spring, c) an image of a CSD performed by one of the hand-reared females in June 2008. Eight of ten females (2008); and eleven of twelve females (2010) gave CSDs, supporting the validity of the video-training method. d) an image of a female displaying in response to song elicited in the operant assay.

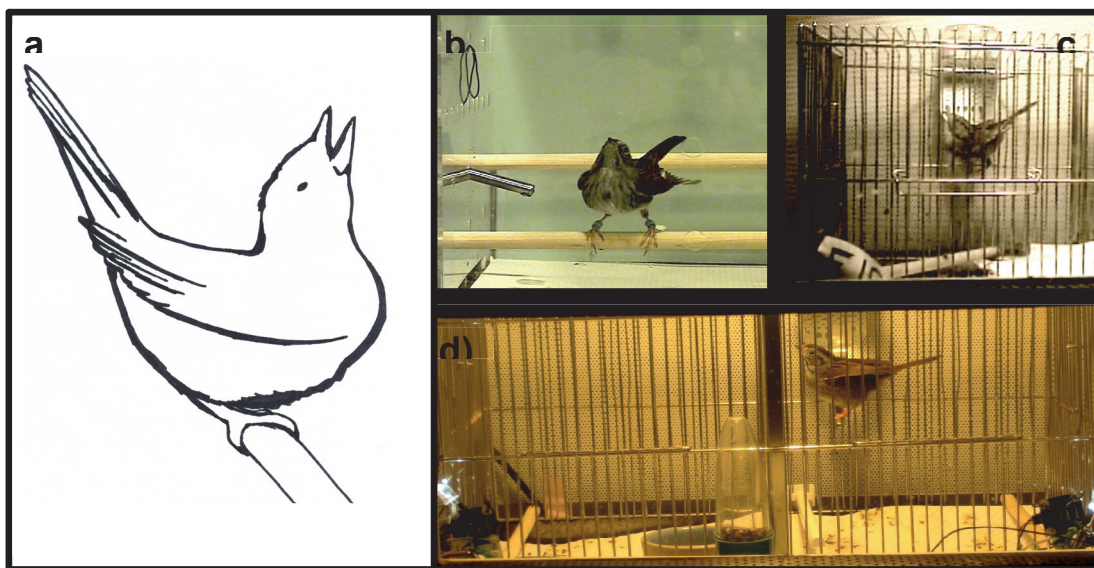




Figure 3. 2 Example song spectrograms of stimuli used in Exp. 1: tutor songs versus novel songs. Tutor stimuli (a,b) were used in song model training and testing, and novel stimuli (c,d) were not experienced previous to testing. In Exp. 1, each female received two out of the ten tutor songs in testing and a contrasting set of two novel songs, which were matched for trill rate and notes/syllable. Each female received a unique combination of tutor and novel song types.

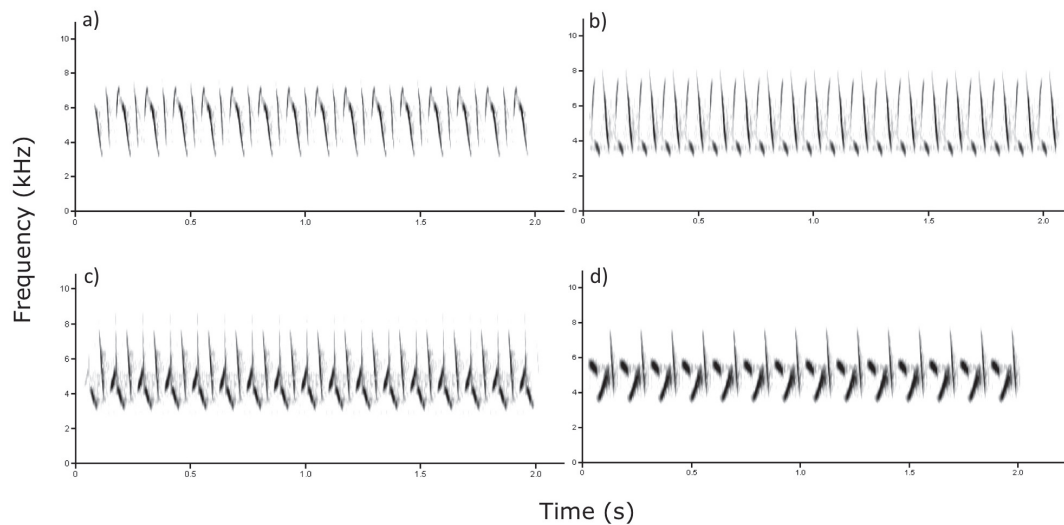


Figure 3. 3 Example spectrograms of stimuli used in Exp. 2: performance versus experience. Three different songs types are pictured at high performance (a-c), control performance (d-f), and low performance (g-h). During song model training, tutor songs were presented at control-performance levels (d-f). The construction of high and low performance was accomplished by increasing or decreasing trill rate. In testing, females received three song stimuli, and each female received a unique combination of three song types. Females were tested with the same song types at high-, normal-, and low-performance levels.

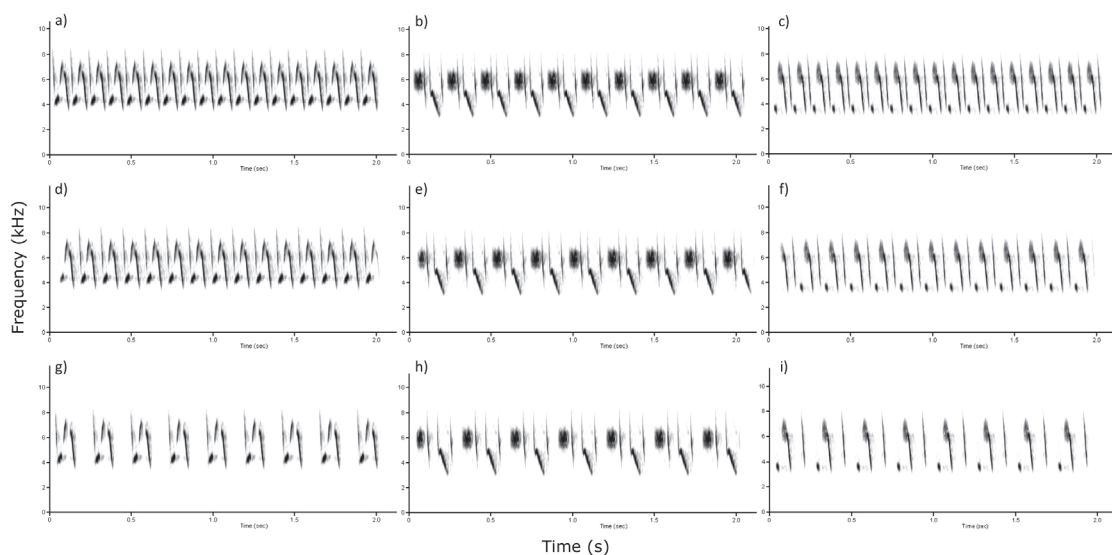
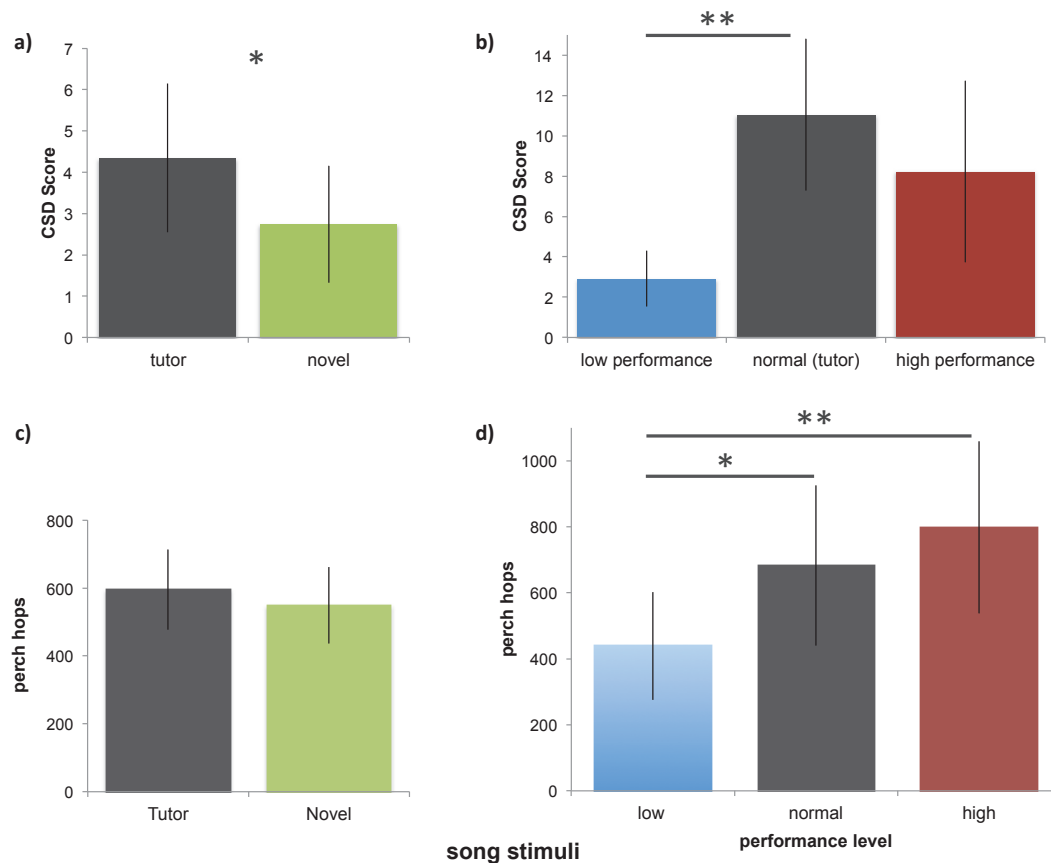


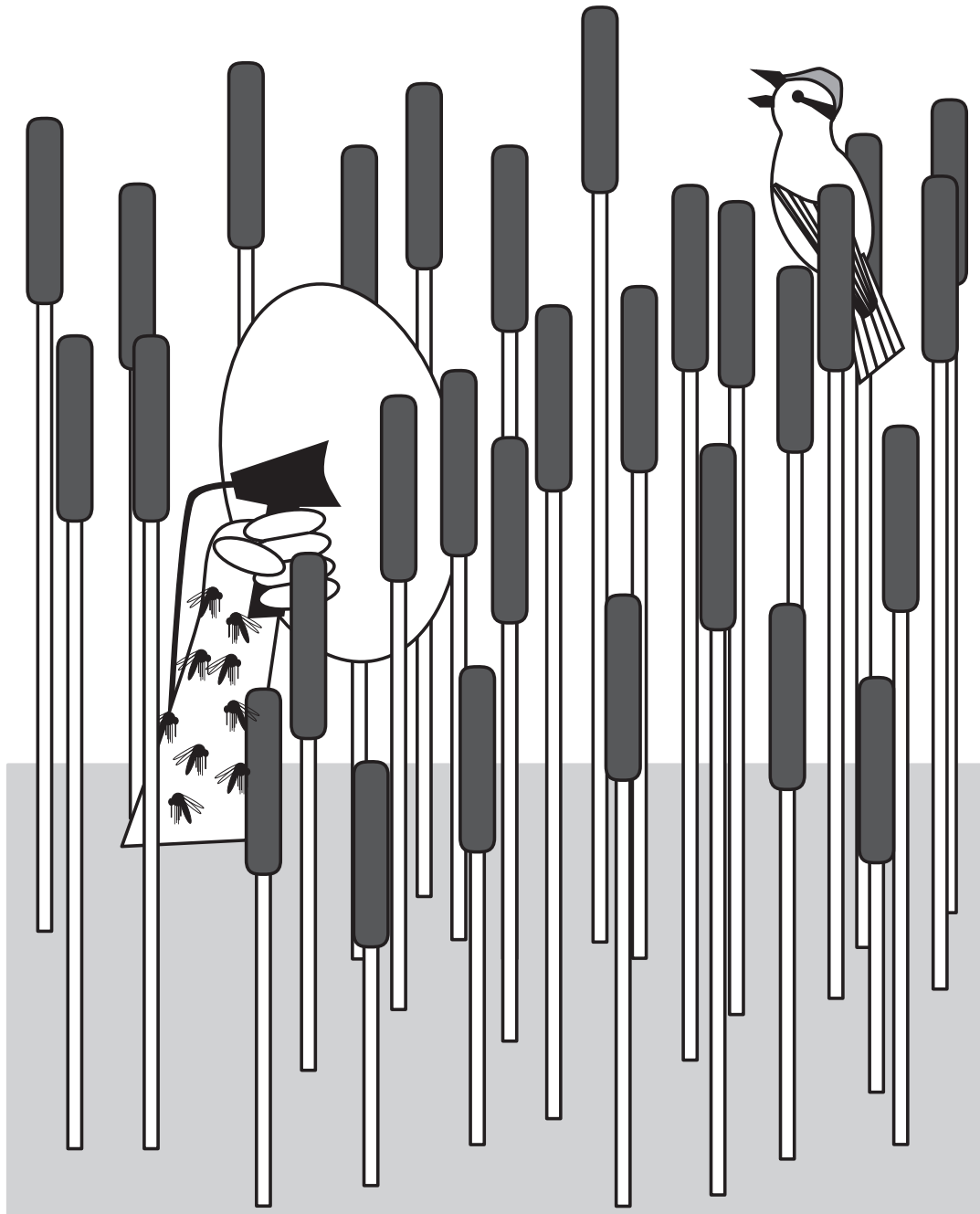
Figure 3. 4 Means and standard errors of female preference behavior with the CSD (a,b) and operant (c,d) assays in two experiments. Experiment 1: (Left panels) Females gave significantly more CSDs in response to their tutor songs than to novel songs, indicating an influence of early experience on adult mate preference (a). While the direction of responses was maintained in the operant assay, the difference between responses did not achieve statistical significance (c). Experiment 2: (Right panels) Females responded most strongly to normal performance, and this value was significant over low-performance versions, but not significant when compared to high-performance versions ( $P > 0.1$ ) with the CSD assay (b). In the operant assay, females elicited songs of normal and high performance significantly more than low-performance versions (d). Again responses to normal and high performance were similar. Symbols indicate levels of significance \* =  $p < 0.05$ , \*\* =  $p < 0.025$  for Wilcoxon matched-pairs sign-ranks tests.



APPENDIX

ILLUSTRATION OF SELECTED QUOTATION II.

CARTOON BY BEN TAFT



"Yes; though you may think me perverse, if it were proposed to me to dwell in the neighborhood of the most beautiful garden that ever human art contrived, or else of a dismal swamp, I should certainly decide for the swamp." – Henry David Thoreau

## BIBLIOGRAPHY

- Aguilar, T. M., R. Maia, E. S. A. Santos, and R. H. Macedo. 2008. Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. *Behavioral Ecology* 19:292-301.
- Anderson, R. C. 2009. Operant conditioning and copulation solicitation display assays reveal a stable preference for local song by female swamp sparrows *Melospiza georgiana*. *Behavioral Ecology and Sociobiology* 64:215-223.
- Andersson, M. 1994. *Sexual Selection*. Princeton University Press; Princeton, NJ.
- Arcese, P. and J. N. M. Smith. 1985. Phenotypic correlates and ecological consequences of dominance in song sparrows. *Journal of Animal Ecology* 54:817-830.
- Baker, M. C., K. J. Spillnabors, and D. C. Bradley. 1981. Early Experience Determines Song Dialect Responsiveness of Female Sparrows. *Science* 214:819-821.
- Bakker, T. C. M. and M. Milinski. 1991. Sequential female choice and the previous male effect in sticklebacks. *Behavioral Ecology and Sociobiology* 29:205-210.
- Ballentine, B. 2009. The ability to perform physically challenging songs predicts age and size in male swamp sparrows, *Melospiza georgiana*. *Animal Behaviour* 77:973-978.
- Ballentine, B., J. Hyman, and S. Nowicki. 2004. Vocal performance influences female response to male bird song: an experimental test. *Behavioral Ecology* 15:163-168.
- Ballentine, B., W. A. Searcy, and S. Nowicki. 2008. Reliable aggressive signaling in swamp sparrows. *Animal Behaviour* 75:693-703.
- Breden, F. and G. Stoner. 1987. Male Predation Risk Determines Female Preference in the Trinidad Guppy. *Nature* 329:831-833.
- Brenowitz, E. A. and M. D. Beecher. 2005. Song learning in birds: diversity and plasticity, opportunities and challenges. *Trends in Neurosciences* 28:127-132.
- Brenowitz, E. A., K. Lent, and D. E. Kroodsma. 1995. Brain space for learned song in birds develops independently of song learning. *Journal of Neuroscience* 15:6281-6286.
- Brenowitz, E. A., D. Margoliash, and K. W. Nordeen. 1997. An introduction to birdsong and the avian song system. *Journal of Neurobiology* 33:495-500.
- Brenowitz, E. A., B. Nalls, J. C. Wingfield, and D. E. Kroodsma. 1991. Seasonal-Changes in Avian Song Nuclei without Seasonal-Changes in Song Repertoire. *Journal of Neuroscience* 11:1367-1374.
- Buchanan, K. L., S. Leitner, K. A. Spencer, A. R. Goldsmith, and C. K. Catchpole. 2004. Developmental stress selectively affects the song control nucleus HVC in the zebra finch. *Proceedings of the Royal Society of London Series B-Biological Sciences* 271:2381-2386.

- Buchanan, K. L., K. A. Spencer, A. R. Goldsmith, and C. K. Catchpole. 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proceedings of the Royal Society of London Series B-Biological Sciences* 270:1149-1156.
- Burt, J. M., S. E. Campbell, and M. D. Beecher. 2001. Song type matching as threat: a test using interactive playback. *Animal Behaviour* 62:1163-1170.
- Byers, J., E. Hebets, and J. Podos. 2010. Female mate choice based upon male motor performance. *Animal Behaviour* 79:771-778.
- Caro, S. P., K. B. Sewall, K. G. Salvante, and K. W. Sockman. 2010. Female Lincoln's sparrows modulate their behavior in response to variation in male song quality. *Behavioral Ecology* 21:562-569.
- Catchpole, C. and P. Slater. 2008. *Bird Song*. University Press, Cambridge.
- Catchpole, C. K., J. Dittami, and B. Leisler. 1984. Differential Responses to Male Song Repertoires in Female Songbirds Implanted with Estradiol. *Nature* 312:563-564.
- Collins, S. 2004. Vocal fighting and flirting: the functions of birdsong. Pp. 39-79 in P. Marler, and H. Slabbekoorn, eds. *Nature's Music: the Science of Bird Song*. Elsevier, San Diego, CA.
- \_\_\_\_\_. 1995. The effect of recent experience on female choice in zebra finches. *Animal Behaviour* 49:479-486.
- Coltman, D. W., M. Festa-Bianchet, J. T. Jorgenson, and C. Strobeck. 2002. Age-dependent sexual selection in bighorn rams. *Proceedings of the Royal Society B-Biological Sciences* 269:165-172.
- Cramer, E. R. A. and J. J. Price. 2007. Red-winged blackbirds *Agelaius phoeniceus* respond differently to song types with different performance levels. *Journal of Avian Biology* 38:122-127.
- Crothers, L., E. Gering, and M. Cummings. 2011. Aposematic Signal Variation Predicts Male-Male Interactions in a Polymorphic Poison Frog. *Evolution* 65:599-605.
- Darwin, C. 1871. *The Descent of Man and Selection in Relation to Race*. John Murray, London.
- de Kort, S. R., E. R. B. Eldermire, E. R. A. Cramer, and S. L. Vehrencamp. 2009. The deterrent effect of bird song in territory defense. *Behavioral Ecology* 20:200-206.
- de Kort, S. R., E. R. B. Eldermire, S. Valderrama, C. A. Botero, and S. L. Vehrencamp. 2009. Trill consistency is an age-related assessment signal in banded wrens. *Proceedings of the Royal Society B-Biological Sciences* 276:2315-2321.

- Derryberry, E. P., N. Seddon, S. Claramunt, J. A. Tobias, A. Baker, A. Aleixo, and R. T. Brumfield. 2012. Correlated Evolution of Beak Morphology and Song in the Neotropical Woodcreeper Radiation. *Evolution* 66:2784-2797.
- Draganoiu, T. I., L. Nagle, and M. Kreutzer. 2002. Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. *Proceedings of the Royal Society of London Series B-Biological Sciences* 269:2525-2531.
- DuBois, A. L., S. Nowicki, and W. A. Searcy. 2009. Swamp sparrows modulate vocal performance in an aggressive context. *Biology Letters* 5:163-165.
- \_\_\_\_\_. 2011. Discrimination of vocal performance by male swamp sparrows. *Behavioral Ecology and Sociobiology* 65:717-726.
- Elwood, R. W. and G. Arnott. 2012. Understanding how animals fight with Lloyd Morgan's canon. *Animal Behaviour* 84:1095-1102.
- Endler, J. A., A. Basolo, S. Glowacki, and J. Zerr. 2001. Variation in response to artificial selection for light sensitivity in guppies (*Poecilia reticulata*). *American Naturalist* 158:36-48.
- Enquist, M. 1985. Communication During Aggressive Interactions with Particular Reference to Variation in Choice of Behavior. *Animal Behaviour* 33:1152-1161.
- Falls, J. B., J. R. Krebs, and P. K. McGregor. 1982. Song Matching in the Great Tit (*Parus-Major*) - the Effect of Similarity and Familiarity. *Animal Behaviour* 30:997-1009.
- Fisher, R. 1930. *The Genetical Theory of Natural Selection*, Clarendon, Oxford.
- Freeberg, T. M., A. P. King, and M. J. West. 2001. Cultural transmission of vocal traditions in cowbirds (*Molothrus ater*) influences courtship patterns and mate preferences. *Journal of Comparative Psychology* 115:201-211.
- Fuller, R. C., D. Houle, and T. J. 2005. Synthesis: Sensory Bias as an Explanation for the Evolution of Mate Preferences. *The American Naturalist* 166:437-446.
- Galef, B. G. and K. N. Laland. 2005. *Social Learning in Animals: Empirical Studies and Theoretical Models*. Bioscience 55:489-499.
- Galef, B. G. and D. J. White. 2000. Evidence of social effects on mate choice in vertebrates. *Behavioural Processes* 51:167-175.
- Hebets, E. A. 2003. Subadult experience influences adult mate choice in an arthropod: Exposed female wolf spiders prefer males of a familiar phenotype. *Proceedings of the National Academy of Sciences of the United States of America* 100:13390-13395.
- Hebets, E. A. and C. J. Vink. 2007. Experience leads to preference: experienced females prefer brush-legged males in a population of syntopic wolf spiders. *Behavioral Ecology* 18:1010-1020.

- Hernandez, A. M., L. S. Phillmore, and S. A. MacDougall-Shackleton. 2008. Effects of learning on song preferences and Zenk expression in female songbirds. *Behavioural Processes* 77:278-284.
- Hof, D. and N. Hazlett. 2010. Low-amplitude song predicts attack in a North American wood warbler. *Animal Behaviour* 80:821-828.
- . 2012. Mortal combat: an apparent intraspecific killing by a male Black-capped Chickadee. *Journal of Field Ornithology* 83:290-294.
- Hughes, M. 1996. Size assessment via a visual signal in snapping shrimp. *Behavioral Ecology and Sociobiology* 38:51-57.
- Illes, A. E., M. L. Hall, and S. L. Vehrencamp. 2006. Vocal performance influences male receiver response in the banded wren. *Proceedings of the Royal Society B-Biological Sciences* 273:1907-1912.
- Irschick, D. J., J. J. Meyers, J. F. Husak, and J. F. Le Galliard. 2008. How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evolutionary Ecology Research* 10:177-196.
- Janicke, T., S. Hahn, M. S. Ritz, and H. U. Peter. 2008. Vocal performance reflects individual quality in a nonpasserine. *Animal Behaviour* 75:91-98.
- Janik, V. M. and P. J. B. Slater. 2000. The different roles of social learning in vocal communication. *Animal Behaviour* 60:1-11.
- Kempnaers, B., G. R. Verheyren, and A. A. Dhondt. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behavioral Ecology* 8:481-492.
- King, A. P. and M. J. West. 1977. Species Identification in North American Cowbird - Appropriate Responses to Abnormal Song. *Science* 195:1002-1004.
- Kirkpatrick, M. 1982. Sexual Selection and the Evolution of Female Choice. *Evolution* 36:1-12.
- Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. *Proceedings of the Royal Society B-Biological Sciences* 270:653-664.
- Kroodsma, D. E. 1978. Aspects of Learning in the Ontogeny of Bird Song. Pp. 215-230 in G. M. B. M. Bekoff, ed. *The Development of Behavior: Comparative and Evolutionary Aspects*. Garland.
- Lahti, D. C., D. L. Moseley, and J. Podos. 2011. A Tradeoff Between Performance and Accuracy in Bird Song Learning. *Ethology* 117:802-811.
- Lailvaux, S. P. and D. J. Irschick. 2006. A functional perspective on sexual selection: insights and future prospects. *Animal Behaviour* 72:263-273.

- Laland, K. N. 1994. Sexual selection with a culturally transmitted mating preference. *Theoretical Population Biology* 45:1-15.
- Lauay, C., N. M. Gerlach, E. Adkins-Regan, and T. Devoogd. 2004. Female zebra finches require early song exposure to prefer high-quality song as adults. *Animal Behaviour* 68:1249-1255.
- Liu, I. A., B. Lohr, B. Olsen, and R. Greenberg. 2008. Macrogeographic vocal variation in subspecies of Swamp Sparrow. *Condor* 110:102-109.
- MacDonald, I. F., B. Kempster, L. Zanette, and S. A. MacDougall-Shackleton. 2006. Early nutritional stress impairs development of a song-control brain region in both male and female juvenile song sparrows (*Melospiza melodia*) at the onset of song learning. *Proceedings of the Royal Society B-Biological Sciences* 273:2559-2564.
- Marler, P. 1976. An ethological theory of origin of vocal learning. *Annals of the New York Academy of Sciences* 280:386-395.
- Marler, P. and S. Peters. 1977. Selective Vocal Learning in a Sparrow. *Science* 198:519-521.
- \_\_\_\_\_. 1981. Sparrows Learn Adult Song and More from Memory. *Science* 213:780-782.
- \_\_\_\_\_. 1982. Structural-Changes in Song Ontogeny in the Swamp Sparrow *Melospiza georgiana*. *Auk* 99:446-458.
- \_\_\_\_\_. 1982. Subsong and plastic song: their role in the vocal learning process. Pp. 25-50 in D. E. Kroodsma, and E. H. Miller, eds. *Acoustic communication in birds*. Academic Press, New York.
- \_\_\_\_\_. 1987. A Sensitive Period for Song Acquisition in the Song Sparrow, *Melospiza melodia* - a Case of Age-Limited Learning. *Ethology* 76:89-100.
- Marler, P. and R. Pickert. 1984. Species-Universal Microstructure in the Learned Song of the Swamp Sparrow (*Melospiza georgiana*). *Animal Behaviour* 32:673-689.
- Maynard-Smith, J. and D. Harper. 2003. *Animal Signals*. Oxford University Press, Oxford, UK.
- Maynard-Smith, J. and G. A. Parker. 1976. Logic of Asymmetric Contests. *Animal Behaviour* 24:159-175.
- Maynard-Smith, J. and G. R. Price. 1973. Logic of Animal Conflict. *Nature* 246:15-18.
- Meitzen, J., C. K. Thompson, H. Choi, D. J. Perkel, and E. A. Brenowitz. 2009. Time course of changes in Gambel's white-crowned sparrow song behavior following transitions in breeding condition. *Hormones and Behavior* 55:217-227.
- Mennill, D. J., L. M. Ratcliffe, and P. T. Boag. 2002. Female eavesdropping on male song contests in songbirds. *Science* 296:873-873.



- Moseley, D. L., D. C. Lahti, and J. Podos. 2013. Responses to song playback vary with the vocal performance of both signal senders and receivers. *Proceedings of the Royal Society B-Biological Sciences* 280(1768).
- Nelson, D. A. 2000. Song overproduction, selective attrition and song dialects in the white-crowned sparrow. *Animal Behaviour* 60:887-898.
- Nowicki, S., S. Peters, and J. Podos. 1998. Song learning, early nutrition and sexual selection in songbirds. *American Zoologist* 38:179-190.
- Nowicki, S., W. A. Searcy, M. Hughes, and J. Podos. 2001. The evolution of bird song: male and female response to song innovation in swamp sparrows. *Animal Behaviour* 62:1189-1195.
- Nowicki, S., W. A. Searcy, and S. Peters. 2002. Quality of song learning affects female response to male bird song. *Proceedings of the Royal Society of London Series B-Biological Sciences* 269:1949-1954.
- \_\_\_\_\_. 2002. Brain development, song learning and mate choice in birds: a review and experimental test of the "nutritional stress hypothesis". *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* 188:1003-1014.
- Peake, T. M., A. M. R. Terry, P. K. McGregor, and T. Dabelsteen. 2002. Do great tits assess rivals by combining direct experience with information gathered by eavesdropping? *Proceedings of the Royal Society B-Biological Sciences* 269:1925-1929.
- Piper, W. H. and R. H. Wiley. 1989. Correlates of dominance in wintering white-throated sparrows - age, sex, and location. *Animal Behaviour* 37:298-310.
- Podos, J. 1996. Motor constraints on vocal development in a songbird. *Animal Behaviour* 51:1061-1070.
- \_\_\_\_\_. 1997. A performance constraint on the evolution of trilled vocalizations in a songbird family (Passeriformes:Emberizidae). *Evolution* 51:537-551.
- \_\_\_\_\_. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409:185-188.
- Podos, J., D. C. Lahti, and D. L. Moseley. 2009. Vocal Performance and Sensorimotor Learning in Songbirds. Pp. 159-195. *Advances in the Study of Behavior*, Vol 40.
- Podos, J. and S. Nowicki. 2004. Performance limits on birdsong production. Pp. 318-342 in P. Marler, and H. Slabbekoorn, eds. *Nature's Music: The Science of Bird Song*. Academic Press, New York.
- Podos, J., S. Nowicki, and S. Peters. 1999. Permissiveness in the learning and development of song syntax in swamp sparrows. *Animal Behaviour* 58:93-103.

- Podos, J., S. Peters, and S. Nowicki. 2004. Calibration of song learning targets during vocal ontogeny in swamp sparrows, *Melospiza georgiana*. *Animal Behaviour* 68:929-940.
- Prather, J. F., S. Nowicki, R. C. Anderson, S. Peters, and R. Mooney. 2009. Neural correlates of categorical perception in learned vocal communication. *Nature Neuroscience* 12:221-228.
- Prather, J. F., S. Peters, S. Nowicki, and R. Mooney. 2008. Precise auditory-vocal mirroring in neurons for learned vocal communication. *Nature* 451:305-U302.
- \_\_\_\_\_. 2010. Persistent Representation of Juvenile Experience in the Adult Songbird Brain. *Journal of Neuroscience* 30:10586-10598.
- Riebel, K. 2000. Early exposure leads to repeatable preferences for male song in female zebra finches. *Proceedings of the Royal Society of London Series B-Biological Sciences* 267:2553-2558.
- \_\_\_\_\_. 2003. Developmental influences on auditory perception in female zebra finches - is there a sensitive phase for song preference learning? *Animal Biology* 53:73-87.
- Riebel, K., M. L. Hall, and N. E. Langmore. 2005. Female songbirds still struggling to be heard. *Trends in Ecology & Evolution* 20:419-420.
- Rots, N. Y., J. deJong, J. O. Workel, S. Levine, A. R. Cools, and E. R. DeKloet. 1996. Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. *Journal of Neuroendocrinology* 8:501-506.
- Ryan, M. J. and A. S. Rand. 1990. The Sensory Basis of Sexual Selection for Complex Calls in the Tungara Frog, *Physalaemus pustulosus* (Sexual Selection for Sensory Exploitation). *Evolution* 44:305-314.
- Sandell, M. and H. G. Smith. 1991. Dominance, prior occupancy, and winter residency in the great tit (*Parus major*). *Behavioral Ecology and Sociobiology* 29:147-152.
- Schmidt, K. L., Moore, S. D., MacDougall-Shackleton, E. A., & MacDougall-Shackleton, S. A. 2013. Early-life stress affects song complexity, song learning and volume of the brain nucleus RA in adult male song sparrows. *Animal Behaviour* 86:25-35.
- Schmidt, R., H. P. Kunc, V. Amrhein, and M. Naguib. 2008. Aggressive responses to broadband trills are related to subsequent pairing success in nightingales. *Behavioral Ecology* 19:635-641.
- Searcy, W. A. 1992. Measureing responses of female birds to male song. Pp. 175-189 in P. McGregor, ed. *Playback and Studies of Animal Communication*. Plenum Press, New York.
- Searcy, W. A., R. C. Anderson, and S. Nowicki. 2006. Bird song as a signal of aggressive intent. *Behavioral Ecology and Sociobiology* 60:234-241.

- Searcy, W. A. and E. A. Brenowitz. 1988. Sexual Differences in Species Recognition of Avian Song. *Nature* 332:152-154.
- Searcy, W. A. and S. Nowicki. 2005. *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems (Monographs in Behavior and Ecology)*. Princeton University Press, Princeton, NJ.
- Searcy, W. A., S. Peters, S. Kipper, and S. Nowicki. 2010. Female response to song reflects male developmental history in swamp sparrows. *Behavioral Ecology and Sociobiology* 64:1343-1349.
- Searcy, W. A. and K. Yasukawa. 1996. Song and Female Choice. Pp. 454-473 in D. Kroodsma, and E. Miller, eds. *Ecology and evolution of acoustic communication in birds*. Cornell University Press, Ithica, NY.
- Smith, S. M. 1984. Flock switching in chickadees - why be a winter floater. *American Naturalist* 123:81-98.
- Soma, M., M. Takahasi, M. Ikebuchi, H. Yamada, M. Suzuki, T. Hasegawa, and K. Okanoya. 2006. Early rearing conditions affect the development of body size and song in Bengalese finches. *Ethology* 112:1071-1078.
- Spencer, K. A., K. L. Buchanan, A. R. Goldsmith, and C. K. Catchpole. 2003. Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Hormones and Behavior* 44:132-139.
- . 2004. Developmental stress, social rank and song complexity in the European starling (*Stumus vulgaris*). *Proceedings of the Royal Society of London Series B-Biological Sciences* 271:S121-S123.
- Suthers, R. A., E. Vallet, and M. Kreutzer. 2012. Bilateral coordination and the motor basis of female preference for sexual signals in canary song. *Journal of Experimental Biology* 215:2950-2959.
- Suthers, R. A. and S. A. Zollinger. 2004. Producing song - The vocal apparatus. Pp. 109-129. *Behavioral Neurobiology of Birdsong*.
- ten Cate, C. and D. R. Vos. 1999. Sexual imprinting and evolutionary processes in birds: A reassessment. Pp. 1-31. *Advances in the Study of Behavior*, Vol. 28.
- Vallet, E. and M. Kreutzer. 1995. Female Canaries Are Sexually Responsive to Special Song Phrases. *Animal Behaviour* 49:1603-1610.
- Verzijden, M. N., C. ten Cate, M. R. Servedio, G. M. Kozak, J. W. Boughman, and E. I. Svensson. 2012. The impact of learning on sexual selection and speciation. *Trends in Ecology & Evolution* 27:511-519.
- Vu, E. T., M. E. Mazurek, and Y. C. Kuo. 1994. Identification of a Forebrain Motor Programming Network for the Learned Song of Zebra Finches. *Journal of Neuroscience* 14:6924-6934.

- Wasserman, F. E. and J. A. Cigliano. 1991. Song Output and Stimulation of the Female in White-Throated Sparrows. *Behavioral Ecology and Sociobiology* 29:55-59.
- West-Eberhard, M. 1984. Sexual selection, competitive communication and species-specific signals in insects. Pp. 283-324 *in* T. Lewis, ed. *Insect communication*. Academic Press, New York.
- \_\_\_\_\_. 2003. *Developmental plasticity and evolution*. Oxford University Press.
- Yu, A. C. and D. Margoliash. 1996. Temporal hierarchical control of singing in birds. *Science* 273:1871-1875.
- Zann, R. and E. Cash. 2008. Developmental stress impairs song complexity but not learning accuracy in non-domesticated zebra finches (*Taeniopygia guttata*). *Behavioral Ecology and Sociobiology* 62:391-400.